METHOD #35

DETERMINATION OF THE ENAMEL SOLUBILITY REDUCTION BY A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the reduction in acid solubility of a hydroxyapatite disc after pretreatment with a dentifrice extract. This procedure is based on a method described by G. C. Forward in Caries Research 11,9,1977.

Reagents	Suggested Type or Source
Chloroform	Baker Analyzed Reagent
Hydroxyapatite	Baker Analyzed Reagent
Calcium Nitrate	Baker Analyzed Reagent
Di-ammonium Hydrogen Phosphate	Baker Analyzed Reagent
Calcium Phosphate	Baker Analyzed Reagent
Potassium Acetate	Baker Analyzed Reagent
Thymol	Baker U.S.P.
Polyethylene Beakers	S.G.A.
Deionized Water	
200 - 400 Silicon Carbide Paper	Union Carbide
Cold Mounting Resin	Robinson Brothers Inc.
Polyethylene Powder	Allied Chemical Co.

Apparatus	Suggested Type or Source
Disc Dissolution Apparatus	See Caries Research 11,9,1977
Magnetic Stirrer	Labline "Magna-Stir"
Hydraulic Ring Press	RIIC
Spectrophotometer	Beckman Instrument Corp.
Atomic Absorption	Beckman Instrument Corp.

Procedure

Preparation of Hydroxyapatite Disc

1) Prepared by suspending polyethylene powder (4.0 gm) in chloroform (50 ml) with magnetic stirring.

2) Hydroxyapatite (36 gm) is added, mixed intermittently for 5 min., filtered and then the mixture dried at 50°C overnight.

3) The dried mixture is sieved (160 mesh) and 0.20 gm sample compressed under vacuum in an RIIC 1.3 cm standard vacuum die, using an RIIC hydraulic ring press (00-25) for 30 sec. at 0.5 tons followed by 90 sec. at 5 tons.

4) The resulting disc is heated at 110°C for 2 hrs. and then one face and the sides spray-painted with enamel paint, leaving one surface exposed for dissolution.

Preparation of Enamel Mosaics

1) Caries-free surfaces are cut from molars which had been stored in thymol-saline solution and embedded in an inert resin (cold mounting resin).

2) The mold used is 30 mm diameter.

3) 6 - 12 surfaces are employed in each mosaic.

4) The surface is prepared by rubbing down with grade 200 silicon carbide paper and distilled water until enamel surfaces are visible.

5) This surface is finally prepared with 40 strokes of grade 400 silicon carbide paper to obtain a constant surface.

6) The surface is then etched for 10 sec. in acetate buffer pH 4.65 to remove any small particles of enamel.

Preparation of Discs for Pretreatment

All discs are hydrated in distilled water (50 ml) for 1 min. removing any air bubbles formed on the disc surface with a brush. Excess water is removed with a tissue.

Pretreatment with Solutions and Toothpaste Slurries

1) The prepared disc is immersed in test solution (20 ml) for the desired time, usually 1 min.

2) Excess solution is removed by washing with distilled water (10 ml) directly from a wash bottle, followed by immersion of the disc in water (50 ml) for 1 min.

3) The disc is then blotted dry with a tissue and is ready for dissolution.

4) Enamel mosaics are pretreated with solution in a similar fashion.

5) Dentifrice (2 gm) is mixed with water (6 ml) in a plastic beaker using a magnetic stirring bar for 2 min.

6) Pretreatment is performed as described for solution, except that a 6 ml slurry replaces the 20 ml test solution.

Dissolution:

1) For the dissolution, the cell is equilibrated in 100 ml potassium acetate buffer (pH:4.65, 1 min) at 37°C contained in a polyethylene cylinder.

2) Duplicate samples for both calcium and phosphorous measurement are normally taken at the start and end of each experiment.

3) The disc is then secured in the disc holder.

4) Dissolution tests are carried out at 37°C by rotating the propeller at 340 rpm using a constant speed motor.

5) The discs are then tested in the buffer for 1 hr.

6) Enamel mosaics are tested for 8 min. in 20 ml of buffer contained in a plastic tube (diameter 3.8 cm) with round base.

Analysis:

The calcium concentration is measured by atomic absorption. One ml of acetate buffer sample is mixed with one ml of 1% lanthanum chloride dissolved in HCl 0.5 N and compared with similarly prepared standards.

Calculation:

Let A = Calcium dissolving from placebo treated disc.

Let B = Calcium dissolving from fluoride disc.

$$\% \text{ reduction in Acid Dissolution rate} = \frac{(A-B)100}{A}$$

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #36

TITLE: DETERMINATION OF ENAMEL SOLUBILITY REDUCTION

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

f. Sodium Monofluorophosphate/Calcium Carbonate

TEST METHOD #36

DETERMINATION OF ENAMEL SOLUBILITY REDUCTION (ESR)

This method compares the solubility in acid of enamel powder which was treated with a fluoride-containing product to that of enamel powder which was treated with a water control. (1)

Treatment Stages

A. Weigh out (using regular weighing paper) 200 ± 1 mg of enamel (100-200 mesh powdered human dental enamel) for each cream being tested and transfer to 400 ml beakers. Include an enamel sample for water control.

B. Next, prepare the test solutions. Thirty gram dental cream - ninety gram distilled water slurries are prepared in 250 ml. Erlenmeyer flasks and agitated for thirty minutes (by a wrist-action shaker - Burrell Corp. Model BB - #10 speed). The slurries are then transferred to centrifuge tubes and centrifuged for twenty minutes using a Servall super speed angle centrifuge run at approximately twenty-three thousand RPM (powerstat set at 100).

C. Decant the supernatant solutions into 150 ml beakers and adjust the pH to 6.6 ± 0.2 by the dropwise addition of 1.0 molar acetic acid or NaOH.

D. As soon as possible, add seventy-five ml portions of the test solutions (measured in 100 ml graduates) to the enamel samples and seventy-five ml of distilled water to the control sample. Stagger the start of the reactions by one minute intervals.

E. Agitate the treatment systems by swirling (using a wrist-action) for about one-half minute at fifteen minute intervals starting with the actual addition of the test solution to the enamel. Treat the enamel samples for exactly one hour. Thirty seconds prior to the end of the hour, swirl the enamel again, this time tilting the beaker so that the enamel collects at the wall-floor junction.

F. At the end of the treatment time, decant the supernatant solution through a 200 mesh screen (U.S. Standard Sieve Series) and wash the enamel sample onto the screen with the aid of a wash bottle. Each screen is supported by a 5.75" funnel on top of a one liter Erlenmeyer flask. Immediately wash the enamel samples with one liter of distilled water to terminate the treatment.

G. Dry the treated enamel (on the screens) overnight at 37°C.

Etching Stage

A. Remove the treated enamel samples from the oven and allow them to cool for one-half hour.

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(1) Muhler, J. C., Boyd, T. M. and VanHuysen, G., J. Dental Research 29, 182 (1950).

B. Weigh out 50 ± 1 mg of each enamel sample (using regular weighing paper) and transfer to a one hundred twenty-five ml glass-stoppered Erlenmeyer flask. In the meantime allow the agitator water bath (Eberbach Catalog No. 6250), SGA #I 3008 (1973) Incubator Shaker Bath, to reach an equilibrium temperature of 98 ± 0.5°F at an agitation speed of 5½ (about 180 reciprocal strokes/min).

C. Pipet twenty ml of 2.0 molar pH 4.0 sodium acetate buffer (room temperature) into each flask, staggering the start of the additions by two minute intervals. Count the time used to make the buffer delivery as part of the etching time. As soon as the buffer is added, place the flask in the water bath for a total time of forty-five minutes. Filter at the end of the etching time using 12.5 cm Whatman No. 1 filter paper; about 20 ml of filtrate is collected.

D. Pipet twenty-five ml of color-forming solution (for phosphate determination) and five ml of the filtrate (from C) into a one hundred ml volumetric flask. Adjust the level of the flasks with distilled water, shake, and allow to stand for at least two hours.

E. Using a 100 ml volumetric flask prepare a blank of twenty-five ml of color forming solution and seventy-five ml of distilled water.

F. Optical density readings are taken with a (Beckman DU Spectrophotometer) using rectangular cells with a 1 cm path length at a wave-length setting of 460 mu.

G. Calculation of % ESR

$$\% \text{ ESR} = \frac{\text{OD (water control)} - \text{OD (sample)}}{\text{OD (water control)}}$$

Where OD = Optical Density. (F)

BIOLOGICAL TEST METHODS

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #38

DETERMINATION OF ANIMAL CARIES REDUCTION

Recommended for the following systems:

a. Stannous fluoride and insoluble sodium metaphosphate abrasive.
b. Stannous fluoride and silica abrasive.
c. Stannous fluoride and calcium pyrophosphate abrasive.
d. Sodium monofluorophosphate and insoluble sodium metaphosphate abrasive.
e. Sodium monofluorophosphate and insoluble sodium metaphosphate abrasive.
f. Sodium monofluorophosphate dicalcium phosphate abrasive.
g. Sodium monofluorophosphate and alumina abrasive.
h. Sodium monofluorophosphate and silica abrasive.
i. Sodium monofluorophosphate and calcium pyrophosphate abrasive.
j. Sodium monofluorophosphate and calcium carbonate abrasive.

TEST METHOD #38

DETERMINATION OF ANIMAL CARIES REDUCTION

RATIONALE

The rat caries test is a model designed for the study and the prevention of human caries. Although using animals it remains a laboratory test which has many features that are relevant to the carious environment found in human beings. It permits the production of acid from dietary sugars by oral bacteria resident in the oral cavity of the rat, and the acid is harboured in plaque in sheltered regions of the teeth. The prolonged contact-time of the acidic plaque with the tooth enamel causes the loss of mineral phase and the onset of caries. The rat model permits other interactions to take place such as the constant bathing of the teeth in saliva, the replenishment of bacterial inoculum on the teeth from other oral environments and also the dilution and removal of a treatment from a site all of which are difficult to effect in tests carried out in vitro.

The mean pH is higher and the buffering capacity, especially with respect to phosphate, is lower in the rat saliva than in human saliva. Thus, the saliva could regulate the carious challenge differently in the two species. The rat flora is almost certainly modified through the use in the test of the cariogenic diet which contains up to 66% sucrose. This level of sugar is given to ensure the onset of caries over a short period of time. The caries occurs most frequently in the rat fissures which are proportionally larger than their human counterpart.

The treatment administered to the rat is greater than that given to the human subject on a unit body-weight basis; additionally, none of the treatment material is expectorated and so the treatment may have, and in the case of fluorides apparently does have, further benefit by a systemic route after ingestion and absorption through the alimentary tract.

The protocol for the rat test has been determined predominantly to evaluate the activity of fluoride, which reduces caries in human beings and also reduces caries in rats over a much shorter time scale. A judgement of the ability of the test on rats to anticipate the effectiveness of a treatment in human clinical studies rests on the results

obtained after appropriate refinement of the protocol in any study undertaken.

In general, however, it can be said that in our experience similar fluoride-containing products can be compared, and results show a close parallel between availability of fluoride and monofluorophosphate ions.

PROTOCOL

Animals (Management)

The method is basically that described by Konig (Konig, Marthaler, Muhlemann; Dent. Zahn - Mund - Kiefer heilk. 29, 99-128, 1958) but certain minor modifications have been incorporated to achieve closer reproducibility between experiments.

Osborne-Mendel rats are interbred in a closed colony on site, and the animals mated randomly. The same mating pairs are used to produce up to five litters.

The breeding animals are maintained on pelleted UAR No. 113 diet, but for 12 days prior to mating are given the richer diet of UAR No. D.03. (UAR Villemoisson-sur-Orge, cf appendix). After mating the males are returned to the original diet, UAR No.113. Weaning occurs when the progeny are 21-26 days of age (generally 22-24 days old to allow experimental work to start on Mondays), after which the dams are returned to diet UAR No.113.

The animals from one litter are randomly assigned one, or preferably two to each of the various treatments and the total number of animals per treatment is generally between sixteen and twenty. Each animal is weighed, numbered and caged separately in a stainless steel wire cage which restricts coprophagy.

The animals are then given diet MIT 2000 (Keyes & Jordan Archs.oral Biol. 9, 377, 1964) supplemented with 2% Gerval (R) (Lederle Lab. Cyanamid International) or Stephan diet 580 (Stephan, J.dent.Res. 30, 484, 1951) fed ad libitum and water (F<0.2ppm) ad libitum. (It has been our experience that these two diets produce the same caries challenge).

Treatments

Treatment is by dipping a small sable-haired brush (Harriet Hubbard Ayer) into undiluted toothpaste which is applied in a rotating fashion to the left mandible whilst the animal's mouth is held open for 15 seconds. The application is repeated on the right mandible with a fresh quantity of paste. Following the treatment, water and food are withheld for one hour. These procedures are applied twice daily on each work day (5 days) for the first two weeks and once daily for the final week. Total period of treatment is 21 days.

Preparation of Tissues

At the end of the treatment period the animals are killed with chloroform and weighed. The two separate mandibles and the complete upper jaw are then excised and the mandibles defleshed by means of a scalpel and dental chisel after immersion in neutralised formal saline for 48 hours. The molar teeth are mounted in thermoplastic wax and sectioned longitudinally to give five to six sections about 200 - 250 um thick using initially the NMRI type of machine as described in the Art and Science of Dental Caries Research p.300 (Ed. R.S. Harris, New York, Academic Press 1968) and finishing the cut with a scalpel.

Dried sections are stained with Shiff's reagent* for 15 seconds and rinsed with water. The stained sections are laid-out in succession from the lingual to buccal aspects on a microscope slide such that the corresponding fissures are aligned vertically down the slide. The left and right molar teeth are placed side by side.

The sections are fixed with a clear varnish.

Using a microscope at X40 magnification, ten fissures are scored on each slide, three from each of the first molars and the two big fissures on each of the second molars. The most severe type of lesion found in any section of the fissure determines the state of caries in that fissure.

The method of scoring lesion severity is as follows:-

0 No coloration in the enamel or dentine of the fissure.

A Pink coloration in the enamel within the fissure.

T Pink coloration at the enamel-dentine junction but without loss of dentinal material.

B Coloration and loss of material at the enamel-dentine junction. This category is divided into three sub-groups:

- B1 Coloration and slight loss of dentine (darkened area) without a hole through the entire section
- B2 Physical separation of part of the dentine from the enamel and/or increased area of coloration.
- B3 More extensive coloration and/or more extensive separation of the dentine and enamel.

C Loss of enamel material from within the fissure (interpretation here requires particular care because of possible artifacts caused by mechanical vibration during the sectioning process).

*0.5g pararosaniline (acridine-free) in 15 ml. N hydrochloric acid + 0.5g potassium metabisulphite in 85 ml. water, decolorised with activated charcoal for 2 minutes after standing for 24 hours. The solution is diluted 1 : 4 with water before use.

At the beginning of an experiment the rats have no caries and at the end of the experiment they have a severity distribution through the various grades of caries. Thus, in computing the severity of caries, the lesions of a particular grade in all the fissures are cumulated with all those lesions of greater severity also, on the premise that lesions progress through the states 0, A, T, B1, B2, B3 to C.

The following example demonstrates the effect. The ten fissures of the mandible have been assessed for caries severity as shown on the first two lines in the Table. These have been totalled on the third line and the cumulated total for each grade is given on the last line. Thus, if one is considering the B1 lesions the value 7 would be used, whereas for B2 it would be only 3.

	0	A	T	B1	B2	B3	C
Left mandible	0	0	2	1	2	0	0
Right "	0	0	1	2	1	0	1
Total lesions	0	0	3	3	3	0	1
Cumulative total:	10	10	10	7	4	1	1

The mean value for each type of lesion over all the rats within one treatment is determined. In our experience, on evaluating toothpastes containing up to about 1000 ppm F, the useful cumulative indices have been the (T + B1 + B2 + B3 + C) lesions, the (B1 + B2 + B3 + C) lesions and the (B2 + B3 + C) lesions. Each of these indices is analysed statistically on a two-way ANOVA for litter and treatment.

Statistical Analysis

The maximum separation of lesions between treatments in a particular severity scale is ten, i.e. the number of fissures scored. For the comparison of treatments, it is desirable that they give the greatest difference on an appropriate scale and that the standard deviation between animals within the treatment is small.

Based on more than 25 tests, the difference between treatment means of toothpastes with or without 1000 ppm of fluoride is 1.6 lesions on the (T + B1 + B2 + B3 + C) scale, 3.2 lesions on the (B1 + B2 + B3 + C) scale and 3.4 lesions on the (B2 + B3 + C) scale. The separation of the treatment means required for a significant difference to occur depends upon the number of animals in each group but, in our experience, for 16-20 animals the separation must exceed 0.6 - 0.9, 0.8 - 1.1 and 0.8 - 1.1 lesions on the respective scales to achieve a 95% confidence level. Thus, it is desirable to use the (B1 + B2 + B3 + C) or the (B2 + B3 + C) scales for comparison of our treatments with our protocol. With other conditions, another scale might be more appropriate.

The response of the test to different fluoride concentrations on the various scales is shown in the appendices. From these graphs it can be seen that the increased level of fluoride or monofluorophosphate in the product does progressively reduce the level of caries during the experimental period.

U. A. R.

FICHE TECHNIQUE U. A. R. N° 1,13

Aliment Complet pour Rats et Souris Stérilisable

Bouchon, diam. 20 %, longueur 25 %.
Bouchon, diam. 10 %, longueur 20 %.
Farine.

Ration journalière du Rat : suivant l'âge et le poids de 18 à 25 g. — Eau à volonté.
Ration journalière de la Souris : suivant l'âge et le poids, de 8 à 12 g. — Eau à volonté.

FORMULE

Céréales	59 %
Issues	4,5 %
Tourteaux Expellers	19,5 %
Farine d'origine Animale	15 %
Activateurs de croissance	6 %
Composé Minéral Vitaminisé	5 %

(A. D. groupe B Méthionine).

GARANTIES

Energie productive (en cal/kg)	1.050
Matières sèches	88
Protéines digestibles	20
Humidité	12
Matières Minérales	6,8
Matières Cellulosiques	3
Matières azotées totales	23
Matières Grasses	5,4
Extr. non azoté (sucre, amid.)	49,8
	100

TENEURS EN ACIDES AMINES

Arginine	12.700 mg/kg
Cystine	3.300 »
Lysine	12.500 »
Méthionine (suppl. synth.)	14.400 »
Tryptophane	2.400 »
Glycine	11.700 »

TENEURS des MINÉRAUX TOTAUX

Phosphore	8.500 mg/kg
Calcium	12.700 »
Potassium	6.500 »
Sodium	2.400 »
Magnésium	1.350 »
Manganèse	115 »
Fer	470 »
Cuivre	11 »
Zinc	43 »
Cobalt	0,09 »
Iode	0,60 »

VITAMINES (calculées aux cent kg)

	Apport Nat.	App. Synthét.	TOTAUX
Vitam. A.	21.000 UI	3.960 000 UI	4.190.000 UI
Vitam. D_3	1.000 UI	2.800.000 UI	2.801.000 UI
Vitam. B_1	500 mg	120 mg	620 mg
Vitam. B_2	300 »	900 »	1.200 »
Vitam. PP.	6.000 »	3.000 »	9.000 »
Vitam. B^5	1.300 »	600 »	1.900 »
Vitam. K_1	25 »	450 »	475 »
Vitam. B_{12}	3,5 »	3 »	6,5 »
Vitam. E	2.300 »	5.275 »	7.575 »
Choline	130.000 »	54.000 »	184.000 »
Vitam. B_6	100 »	100 »	200 »
Acide Folique	50 »	0 »	50 »
Biotine	5 »	0 »	5 »

Les vitamines sont calculées largement pour tenir compte des traitements divers de stérilisation.

Cet aliment est destiné aux laboratoires qui possèdent une animalerie E.O.P.S. Sa forme (bouchon de 10 %) permet une stérilisation de l'aliment directement par les méthodes classiques.

Présentation : Sac papier 50 kg et 25 kg.

7, Rue du Maréchal-Galliéni, Villemoisson-sur-Orge (S.-&-O.) -- Tél. 921-13-69

U.A.R.

FICHE TECHNIQUE U. A. R. N° D 03

Aliment Complet Rats et Souris Stérilisable Elevage

Bouchons 15 %.
Farine.

Ration journalière du Rat : suivant l'âge et le poids, de 16 à 22 g. — Eau à volonté.
Ration journalière de la Souris : suivant l'âge et le poids, de 6 à 10 g. — Eau à volonté.

FORMULE

Céréales et Produits Hydrocarbonés	59	%
Tourteaux Expellers	7	%
Farine d'origine Animale	24	%
Activateurs de croissance	5	%
Composé Minéral Vitaminisé (A. D. groupe B).	4	%

ANALYSE MOYENNE

Énergie (en cal kg)	3.600	
Matières sèches	88	
Protéines digestibles	22,4	
Humidité	12	
Matières Minérales	6,1	
Matières Cellulosiques	2,7	
Matières azotées totales	24	
Matières Grasses	8	
Extr. non azoté (sucre, amid.)	47,2	
	100	

TENEURS EN ACIDES AMINES (calculées)

Arginine	12.800	mg/kg
Cystine	3.500	»
Lysine	16 500	»
Méthionine	5.600	»
Tryptophane	2.300	»
Glycine	12.300	»

TENEURS en MINÉRAUX TOTAUX

	App. Nat. (moy.)	App. par C.M.	TOTAUX
			mg kg
Phosphore	6.700	3.500	10.200
Calcium	5.700	4.600	10.300
Potassium	5.900	0	5.900
Sodium	1.700	800	2.500
Magnésium	1.400	137	1.537
Manganèse	29	40	69
Fer	95	150	245
Cuivre	12	15	27
Zinc	23	45	68
Cobalt	0,07	1,5	1,6
Iode	apporté sous forme assimilable par algues marines.		

VITAMINES (calculées aux cent kg)

	App. Nat. (moy.)	App. Synthet.	TOTAUX
Vitamine A	20.000 UI	2.500.000 UI	2.520.000 UI
» D^3	4 500 »	700.000 »	704.500 »
» B^1	700 mg	250 mg	950 mg
» B^2	500 »	1.000 »	1.500 »
» B^5	1.500 »	2.400 »	3.900 »
» B^6	500 »	400 »	900 »
» B^{12}	2,1 »	2,5 »	4,6 »
» E	2.100 UI	10.000 UI	12.100 UI
» K	25 mg	500 mg	525 mg
» PP	5.700 »	3 500 »	9.200 »
Acide Folique	75 »	25 »	100 »
Acide P. A. B.	0 »	50 »	min. 50 »
Biotine	15 »	10 »	25 »
Choline	156.000 »	80.000 »	236 000 »
Meso-Inositol	0 »	100 »	min. 100 »

Les teneurs en vitamines sont indiquées avant stérilisation. Une surcharge par rapport aux besoins palliе les destructions subies pendant la stérilisation.

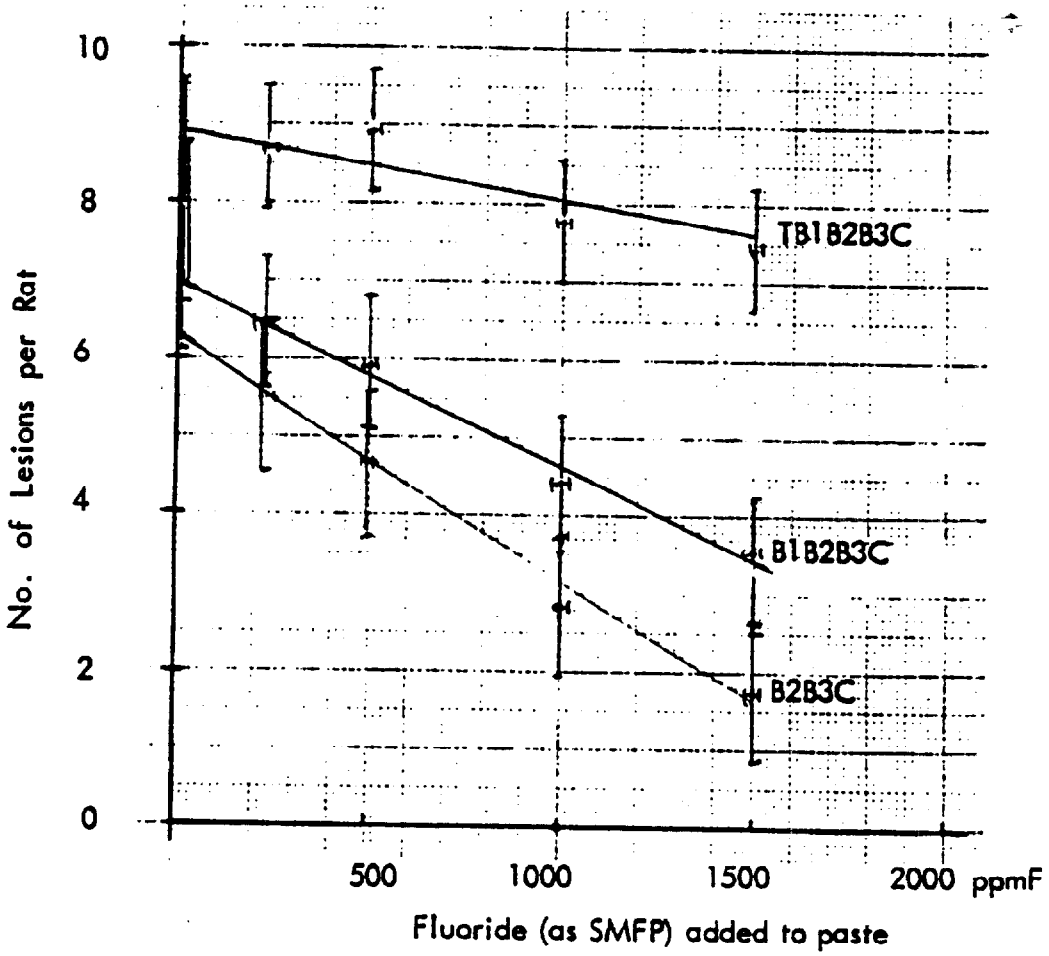
Aliment spécialement conditionné pour permettre une stérilisation directe en sac.

Autres présentations : B 03 - Aliment complet Rats et Souris Autoclavé Elevage.
C 03 - Aliment complet Rats et Souris Stérilisé Elevage.

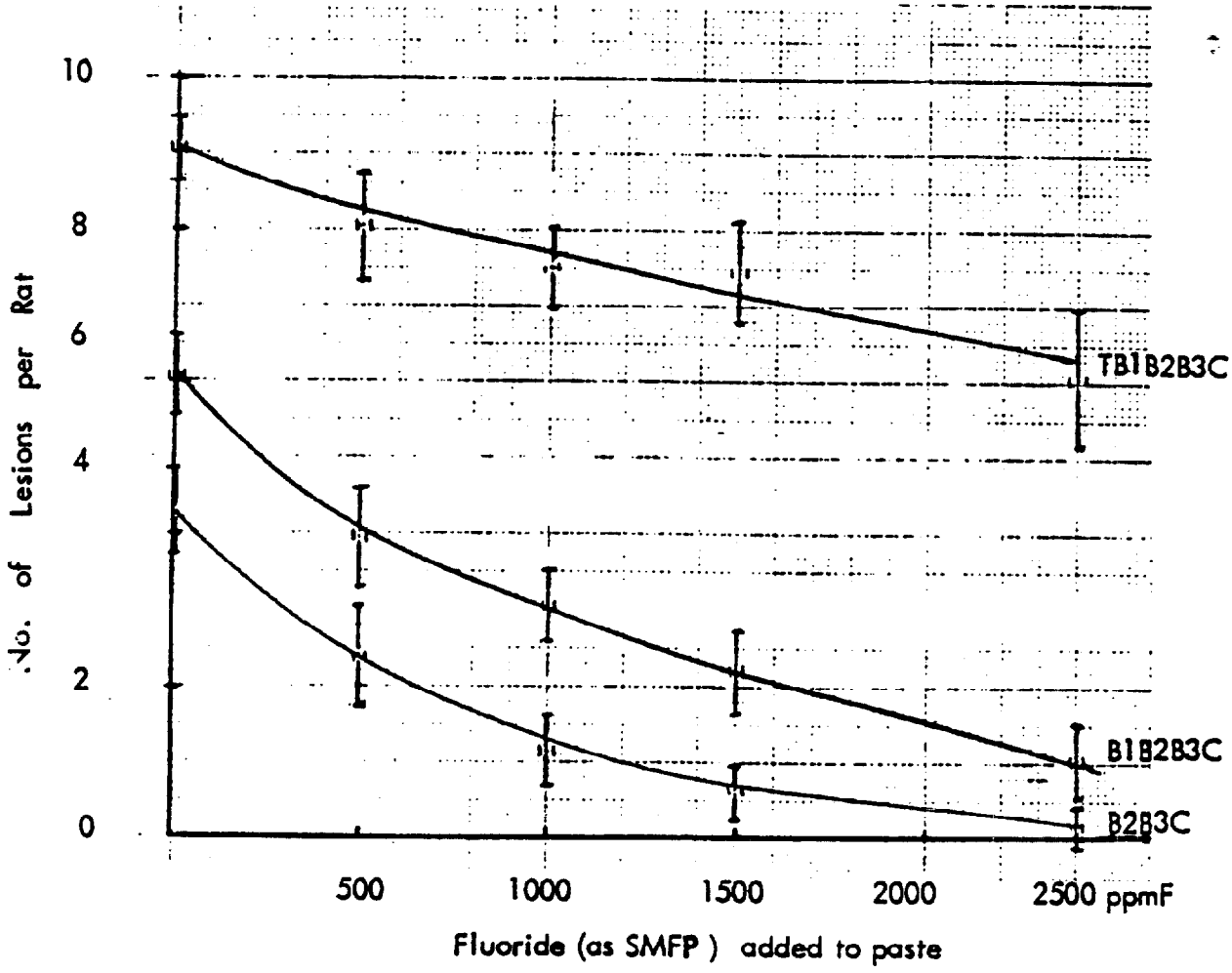
(Imp. Lorot, Epinay)

7, Rue du Maréchal-Galliéni, 91 - Villemoisson-sur-Orge -- Tél. 921-13-69

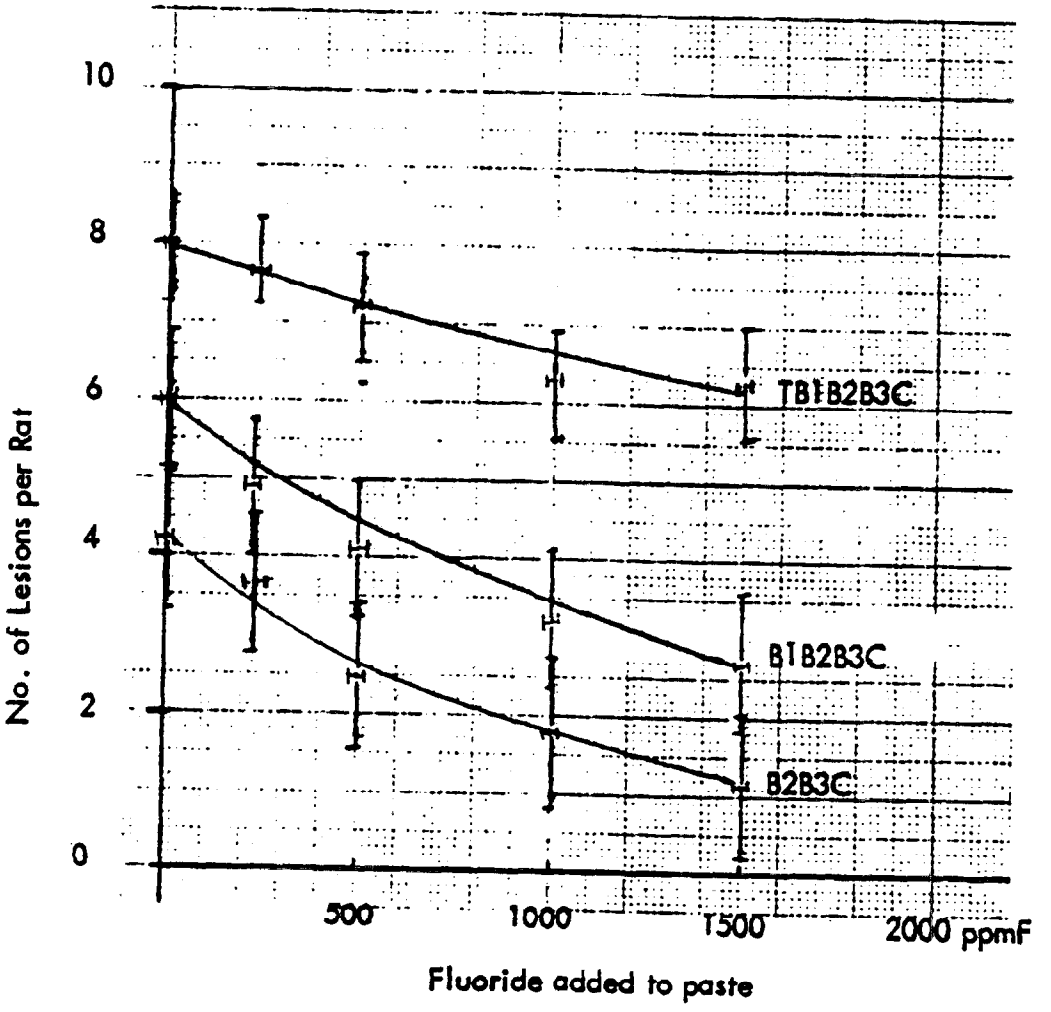
Effect of Fluoride (SMFP) in a Silica Abrasive Toothpaste on the Caries Incidence in Rats on the T + B1 + B2 + B3 + C, B1 + B2 + B3 + C and B2 + B3 + C Scales (showing the 95% Confidence Limits).



Effect of Fluoride (SMFP) in an Alumina Abrasive Toothpaste on the Caries Incidence in Rats on T + B1 + B2 + B3 + C, B1 + B2 + B3 + C and B2 + B3 + C Scales (showing 95% Confidence Limits).



Effect of Fluoride (SnF_2) in a Silica Abrasive Toothpaste on the Caries Incidence in Rats on the T + B1 + B2 + B3 + C, B1 + B2 + B3 + C and B2 + B3 + C Scales (showing 95% Confidence Limits.)



STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #39

TITLE: DETERMINATION OF ANIMAL CARIES

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

f. Sodium Monofluorophosphate/Calcium Carbonate

TEST METHOD # 39

DETERMINATION OF ANIMAL CARIES

Animals and General Procedures

The experiment is conducted on litters of Osborne-Mendel or Cara rats, each litter consisting of sufficient animals to permit having one sibling in each experimental (and control) group. In order to avoid fissure impaction with wood particles, the animals with their dams are transferred on day 13, prior to tooth eruption, from the breeding cages to screen bottom stainless steel cages without bedding and fed finely powdered Nafag* diet. The animals are weaned when 21 days old and transferred to the experimental cages, two in each cage and randomly distributed to the test treatments. On day 22 and 23, the oral flora of the rats is superinfected twice daily with heavy suspensions of Streptococcus mutans OMZ 176, Actinomyces viscosus OMZ 105 and feces from caries active Osborne-Mendel rats.

At weaning, the animals receive diet 2000a (56% sucrose, 28% skim milk, 8% wheat flour, 5% yeast, 2% Gevral Protein Lederle, 1% sodium chloride) and drinking water ad libitum. The animals are sacrificed after 20-25 experimental days. From day 24 onwards the test compounds are applied once daily by means of disposable syringes.

Caries Incidence

Under a dissection microscope the extension of smooth surface lesions on the buccal surfaces of the first and second lower molars is recorded using Keyes' (1) area units E. Afterwards, serial mesio-distal sections are prepared from the mandibles with an internal-rim cutting machine(2). The sections are stained, serially mounted on slides and randomized prior to evaluation. A total of 12 fissures of the first and second lower molars are examined microscopically for carious lesions according to the Zurich method(3).

* Rat checkers No. 184, Nafag AG.

References

(1) Keyes, P. H.: Dental caries in the molar teeth of rats. II. A method of diagnosing and scoring several types of lesions simultaneously. J. Dent. Res. 37, 1088; 1958.

(2) Jansen, M. T.: An improved method for the preparation of "serial" sections of undecalcified dental tissues. J. Dent. Res. 29, 401; 1950.

(3) Konig, K. G.: Moglichkeiten der Kariesprophylaxe beim Menschen und ihre Untersuchung im kurzfristigen Rattenexperiment. Hans Huber, Bern/Stuttgard 1966.

TEST METHOD #37

Title: Determination of Animal Caries Reduction

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate
b. Stannous Fluoride - silica
c. Stannous Fluoride - insoluble sodium metaphosphate
d. Sodium Monofluorophosphate - insoluble sodium metaphosphate
e. Sodium Monofluorophosphate - dicalcium phosphate dihydrate
f. Sodium Monofluorophosphate - alumina
g. Sodium Monofluorophosphate - silica
h. Sodium Monofluorophosphate - calcium pyrophosphate
i. Sodium Monofluorophosphate - calcium carbonate
j. Sodium Fluoride - high-beta-phase calcium pyrophosphate

TEST METHOD #37

Determination of Animal Caries Reduction

Animals (Management)

Wistar rats are obtained from Harlan Industries, Inc., Cumberland, Ind. They are shipped via truck and arrive on Tuesdays at 22-23 days of age. Animals are received in twenty litters of ten randomly sexed animals per litter, thus providing the 200 animals used in a standard rat caries study. One animal from each litter is then randomly allocated to one of the treatment groups and placed in a numbered stainless-steel wire-bottom cage (litter mates occupy the same position in each group; e.g., all animals from litter #1 are allocated to the first cage of each treatment group).

Animals are then weighed in and the weight recorded in the lab book. Animals are fed cariogenic diet #469 ad libitum and deionized water ad libitum.

Treatments

Treatment requires the use of long-stem cotton-tipped swabs. The swab is dipped into a slurry prepared with toothpaste diluted 1:1 (w/v) with deionized water. This dilution is mixed on a "Magnestir" for five minutes prior to treatment application. With the rat's mouth held open by means of a stainless steel retaining clamp, the dipped swab is brushed against the maxillary molars with a front-to-back stroke repeated six times. On the mandible, the swab is dipped into the treatment slurry and then rotated toward the cheek, thereby moving around the tongue to reach the mandible molars. Again six rotations per mandible are required. This procedure is repeated on the opposite side of the mouth with a fresh quantity of toothpaste slurry. Treatment is applied twice daily, beginning the Wednesday after arrival, through Friday. (Saturday and Sunday are not treatment days.) Treatment resumes on the following Monday and goes through Friday with the weekend off, and again resumes on Monday through Friday. The following week the animals are treated on Monday and Tuesday, and a final body weight is obtained.

Preparation of Tissues

On the Wednesday after the final treatement day the animals are sacrificed by decapitation. The tongue is excised and the cheeks incised to the angle of the jaw. A tag with the animal's number (cage number) is attached to the snout of the animal with an 8-inch string. The mouth is propped open with a short piece of Tygon tubing. Once animals from the entire test are sacrificed, the heads are lowered into vats of 2% silver nitrate staining solution for one hour. Upon removal from the stain, the heads are rinsed in at least three changes of running tap water. The heads are then placed in aluminum foil baking pans, the bottom of the pan covered with tap water, and the pan covered loosely with heavy-gauge aluminum foil.

After staining, the aluminum baking pans containing the heads are autoclaved at approximately 120°C and 10 lbs. pressure for 35 minutes, after which the steam is turned off and the pans allowed to stand for another 15 minutes before the heads are removed.

After this procedure the bones containing both upper and lower molars for each rat can easily be lifted from surrounding tissue and placed into the animal's pre-numbered plastic vial for future identification. These vials are left open for 24-36 hours to dry at room temperature and are then closed until they are to be sectioned.

Next all the vials for the test are arranged numerically, and microscope slides are made up with corresponding animal number (one per rat) and the study number attached. Considering one animal at a time, each quadrant is hemi-sectioned longitudinally, and each section is permanently mounted on the microscope slide. Eash quadrant occupies the same position on the slide as in the animal's mouth (e.g., the right upper quadrant is mounted in the right upper corner of the slide).

Grading

Using a microscope at 30X magnification, 22 fissures and 24 smooth surfaces are graded per slide/animal. Each fissure is divided by an imaginary line through the middle of its bottom, and then each side of the fissure is assigned a severity grade. Since each quadrant is sectioned longitudinally, both halves of each quadrant are graded, and the most severe grade is recorded for each corresponding smooth surface or half fissure. In all there are 68 grades per slide/animal.

The method of scoring lesion severity is as follows:

0 - no stain in the enamel or dentin at site.

1 - dark brown stain in enamel only.

2 - dark brown stain in enamel extending to the dentin/enamel junction but no further.

3 - stain through the enamel and into the dentin.

At the beginning of a study one group of rats is sacrificed to obtain a mean zero-time severity score per animal. At the end of the study all treatment groups are sacrificed and graded to obtain a mean severity score per animal. Thus in computing the severity of caries in each particular group, the 68 smooth surface and half fissure grades for each animal in the group are totaled. This number is then divided by the number of animals in the group to obtain the mean severity expressed as mean number of hypomineralized areas ($\bar{x}$ HMA). With all $\bar{x}$ HMA scores tabulated, a percent reduction is calulated for each treatment group. This is done by subtracting the score of the test group from that of the water or placebo control group and then dividing by the water or placebo control group score. This number is expressed as a percent by multiplying by 100. Further statistical analysis is performed to determine significant differences among the groups if they exist. A standard analysis of variance is used. Treatments are ranked by a Newman-Keuls analysis as described by Snedecor and Cochran*.

This method is further described in the following articles:

1. Francis, M.D., Arch. Oral Biol. 11:141-148, 1966.

2. Briner, W.W., and Francis, M.D., Caries Res. 5:180-187, 1971.

3. Donaldson, J.D.; White, W.E.; Briner, W.W.; and Cooley, W.E., J. Dent. Res. 53:648-652, 1974.

*Snedecor, G.W., and Cochran W.G.: Statistical Methods, 6th ed, Ames, Iowa: Iowa University Press, 1967, pp 273-275.

TEST METHOD #40

Determination of Fluoride Uptake by Enamel

To prepare for the exposure of enamel to the dentifrice, sound upper central incisors are scraped free of adhering flesh and thoroughly cleaned using a pumice slurry. The cleaned enamel surface is exposed for thirty seconds to a stirred 2M $HClO_4$ solution. This $HClO_4$ treatment will normally remove a layer of enamel approximately 50 μ thick. Removal of the outer layer of enamel gives an enamel substrate less likely to be contaminated with F^- ion or metallic impurities. A circle, 4mm in diameter, is cut from the labial surface of the incisor with a diamond hollow-core drill.* The circle is cut under water to prevent local overheating.

The dentin attached to the enamel circle is not removed but is ground flat to yield a sample with the shape of a right cylinder. The cirlces are glued to a ½" cast acrylic road approximately 2" long using a two-component epoxy resin. The mounted circles are ground flat with 600-grid silicon carbide on a plastic plate and then polished with 600-grit silicon carbide on wet silicone cloth. All cut surfaces of enamel and dentin are covered with blue inlay wax, leaving only the highly polished enamel surface exposed.

The enamel is etched by mounting the rod in the chuck of a variable speed stirrer and rotating the enamel surface at 150 rpm in a 1-ml portion of 2M $HClO_4$. Two successive etches are taken, the first for thirty seconds and the second for sixty seconds. The etch solutions are diluted 1:1 with TISAB buffer modified by the addition of NaOH to result in a pH of 5.2 after addition of the fluoride sample. The solutions are analyzed for fluoride using a fluoride electrode. This analysis gives the indigenous F^- level before treatement to permit correction of the figures obtained after treatement.

After the two pre-etches the enamel surfaces are again ground flat and polished as described above. Decalcification of the enamel is carried out by exposing the samples for 24 hours at room temperature to a solution of 0.025M lactic acid plus 0.0002 M MHDP** (disodium dihydrogen methanehydroxydiphosphonate). After completion of the decalcification period, the samples are removed from the medium, rinsed with distilled water and stored in a closed jar containing a few drops of water and Roccal.

For the evaluation of dentifrices a supernatant is prepared for treating the decalcified enamel. Supernatants are normally prepared using a ratio of one part dentifrice to three part diluent. The diluent can be either water or pooled saliva. The 3/1 mixture of diluent and dentifrice is mixed thoroughly and the resultant slurry centrifuged for 30 minutes at 11,000 rpm. This procedure will normally produced a clear supernatant free of suspended solids. Treatment of the enamel samples is effected by rotating them slowly (75 rpm) in the supernatant for a fixed time, normally 30 minutes. After the treatment, the samples are rinsed for a few seconds in running distilled water and stored over water prior to etching. The treated samples are etched in $HClO_4$, diluted,

* Horico Diamond Drill; Hopf, Ringleb & Co., Berlin-Lichterfelde, Gardeschutzenweg 82, Germany.

**Monsanto Co., St. Louis, Missouri.

and analyzed for F^- ion as described previously. Fluoride uptake values can be reported as total μg. F^- (corrected for background), μg. F^-/cm^2 enamel, or ppm F^- in the enamel.

Preparation of Decalcification Medium

The decalcification medium consists of 0.025M lactic acid plus $2x10^{-4}M$ Na_2H_2MHDP (disodium dihydrogenmethanehydroxydiphosphonate), adjusted to $pH^2 4.5$. Its exact preparation is as follows:

A stock solution of approximately 1M lactic acid solution at natural pH is aged for at least 50 days at room temperature or for 7 hours in a steam bath. These procedures hydrolyze the lactate polymers to the monomer. After aging, the exact concentration is determined by titration with base. This solution will keep indefinitely.

Sufficient stock 1M lactic acid solution to give 0.025M lactate concentration is mixed with sufficient .002M aqueous stock solution of Na_2H_2MHDP to give a concentration of .0002M, diluted to less than the desired volume, adjusted to pH 4.50 with NaOH, and then brought to final volume. The usual laboratory procedure followed is to weigh 5g of the 1M lactic acid solution into a beaker, and subsequently add 20g of the MHDP solution. The sample is then diluted to 180g with distilled water, and the pH is adjusted to pH 4.5 with a saturated sodium hydroxide solution. Subsequently, the entire sample is brought up to a total weight of 200 g with distilled water. The medium will keep for several days, but bacterial or fungal growth will eventually occur, and then the medium should be discarded. The growth can be inhibited by rinsing all containers with formaldehyde solution before use and/or refrigerating.

TEST METHOD #40

Title: Determination of Fluoride Uptake by Enamel

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate
b. Stannous Fluoride - insoluble sodium metaphosphate
c. Sodium Monofluorophosphate - insoluble sodium metaphosphate
d. Sodium Monofluorophosphate - dicalcium phosphate dihydrate
e. Sodium Monofluorophosphate - alumina
f. Sodium Monofluorophosphate - silica
g. Sodium Monofluorophosphate - calcium pyrophosphate
h. Sodium Monofluorophosphate - calcium carbonate
i. Sodium Fluoride - high-beta-phase calcium pyrophosphate

X

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #41

DETERMINATION OF FLUORIDE UPTAKE

Recommended for the following systems:

a. Stannous fluoride and silica abrasive.
b. Stannous fluoride and calcium pyrophosphate abrasive.
c. Sodium monofluorophosphate and insoluble sodium metaphosphate abrasive.
d. Sodium monofluorophosphate dicalcium phosphate abrasive.
e. Sodium monofluorophosphate and alumina abrasive.
f. Sodium monofluorophosphate and silica abrasive.
g. Sodium monofluorophosphate and calcium pyrophosphate abrasive.
h. Sodium monofluorophosphate and calcium carbonate abrasive.

TEST METHOD #41

DETERMINATION OF FLUORIDE UPTAKE

Principle of Method

The procedure described here is based on the work of McCann et al[1], with some minor modifications. Sound, excised teeth are acid-etched to remove the fluoride rich outer layers of the enamel. This assures that the substrate being examined is principally hydroxyapatite. When the teeth have been etched to a point where the difference between the fluoride contents of successive enamel layers is small, the acid etch solution is analyzed for calcium and/or phosphorus to determine the weight dissolved from the tooth. The etch solution is also analyzed for fluoride present in the dissolved portion of the enamel. A fluoride-containing product (dentifrice, mouthrinse, etc.) is then applied to the teeth, converting a portion of the hydroxyapatite to caries-resistant fluorapatite via uptake of fluoride into the enamel structure. The degree of fluoride incorporation into the enamel is determined by performing another acid etch on the tooth and analyzing the etch solution for the weight of fluoride and weight of enamel. Comparison of the enamel fluoride content following fluoride treatment with that prior to fluoride treatment gives the amount of fluoride taken up by the enamel.

Methods and Materials

The following solutions and equipment are required:

a) 0.5 N $HClO_4$ - Dilute 54 ml of 60% $HClO_4$ (Baker Analyzed Reagents) with deionized water to give 1 liter (volumetric flask).

b) 0.5 M sodium citrate - Weigh 294.1 g of sodium citrate dihydrate (Baker Analyzed Reagent, 1-3646) into a 2-l volumetric flask. Dilute to mark with deionized water.

c) One 10-ml capacity and one 20-ml capacity Repipet dispenser (Labindustries Inc.)

d) 1 oz. polyethylene bottles (Nalgene Cat. No. 2003-0001).

e) Four-dram glass vials (Ace Scientific Cat. No. 10-5032/22).

f) 3.0 ml disposable syringes (Becton Dickinson, Ace Cat. No. 14-4265).

The acid etch and exposure to the test dentifrice slurry (or other fluoride-containing product) should be carried out as follows:

A. Selection and Preparation of Teeth

1. For each product to be tested, twenty noncarious excised teeth (usually molars) ar employed. Remove debris and residual tissue with a dental scaler, cleaning the ro portion of the tooth as well as the crown. Pumice each tooth (flower of pumice) for one minute to assure complete removal of extraneous matter.

2. Cover roots and occlusal surfaces with polyurethane (Varathane, Flecto Company, Oakland, Ca.) to prevent acid etching of these areas. (A minimum of two coats of polyurethane should be used.) As an added measure of protection against acid etching, apply one coat of acid-resistant nail polish to the polyurethane-covered areas and allow to dry.

3. In order to determine whether any calcium, phosphorous, or fluoride contamination from the polyurethane and nail polish layers occurs during acid-etching, blanks are prepared. Coat the ground glass portion of a size 9 glass stopper (volumetric flask) with two layers of polyurethane and one layer of nail polish. Prepare one stopper in this manner for each fluoride product to be tested. Each stopper should now be treated as if it were an actual tooth.

4. Place each tooth and blank in a clean four-dram glass vial. Acid-etch teeth and blanks by dispensing (Repipet) 3.0 ml of 0.5 N $HClO_4$ into the vials and swirling for 1.0 minute. Discard acid etch and rinse tooth and vial with deionized water. Repeat this acid etch for all teeth and blanks to give a total of two one-minute etches.

5. Acid-etch (3.0 ml 0.5 N $HClO_4$) all teeth and blanks for 20 seconds with swirling, discard acid etch and rinse. Repeat this step three more times to give a total of four 20 sec. acid etchings. The difference in fluoride content between successive layers of enamel should now be relatively small.

B. Pretreatment Etch

6. Rinse teeth and blanks with deionized water and dry using a stream of nitrogen. Place each tooth into a 1-oz. Nalgene bottle. Perform the pre-treatment etch by dispensing (Repipet) 3.0 ml of 0.5 N $HClO_4$ into the tooth-containing bottle. Swirl vigorously for 20 seconds. At the 20-second mark, dispense (Repipet) 12.0 ml of 0.5 M sodium citrate into bottle to quench the acid etch. Cap bottle and shake vigorously to mix contents. Transfer the 15.0 ml of etch solution to another 1-oz. Nalgene bottle, cap and save for calcium, phosphorous and fluoride analyses. Brush the etched tooth (toothbrush) lightly under running distilled water. Store the tooth in its labeled four-dram glass vial in deionized water until ready for exposure to the dentifrice slurry or other fluoride treatment.

C. Fluoride Treatment

7. Discard water from glass vials. Gently blow dry tooth (or blank) while still in the vial using a stream of nitrogen. With a 3.0 ml disposable syringe (Becton-Dickinson), dispense 3.0 ml of the fluoride product (25% w/w slurry

in the case of a dentifrice) into the vial. Swirl continuously for 15.0 minutes. At the end of this time, rinse both tooth and vial using distilled water. Lightly brush the tooth under running distilled water, and allow to stand in deionized water (in labeled glass vials) for at least 20 hours. This allows enough time for CaF_2 to slough off the tooth so that only the amount of fluoride taken up as fluorapatite (or $Sn_3F_3PO_4$ and fluorapatite in the case of SnF_2 dentifrices) is determined.

D. Post-Treatment Etch

After 20 hours, again rinse and lightly brush the teeth under running distilled water. Dry the teeth with a nitrogen stream and place into labeled 1 oz. Nalgene bottles. Perform the post-treatment etch in exactly the same manner as the pre-treatment etch (Part B). Save the 15.0 ml of etch solution for calcium, phosphorous and fluoride analysis.

E. Analysis of Etch Solutions

Analysis of calcium, phosphorous and fluoride content of the etch solutions are carried out using the following methods:*

Calcium: Atomic absorption technique.

Phosphorous: Automated colorimetric technique.

Fluoride: Specific ion electrode technique.

F. Determination of Pre- and Post-Treatment Enamel Fluoride Levels

The calcium, phosphorous and fluoride levels for all teeth in a particular fluoride treatment group are adjusted for the calcium, phosphorous and fluoride levels determined for the blank (glass stopper sample) corresponding to that group. Pre- and post-treatment enamel fluoride concentrations are then calculated using one of the following two equations:

a) $Sn_3F_3PO_4$-fluorapatatite equation (the equation of Stearns)[2]

Use this equation when the fluoride agent being tested is stannous fluoride. Here the formation of $Sn_3F_3PO_4$ in the tooth as well as fluorapatite is taken into account:

$$\text{ppm F} = \frac{\text{Weight of fluoride} \times 10^6}{(5.349 \times \text{weight } PO_4^{-3}) - (5.099 \times \text{weight } Ca^{+2})}$$

To use this equation, the calcium, phosphorous and fluoride content of the etch solutions must be determined.

*Refer to Appendix A for a detailed description of these three assays,

b) Fluorapatite equation

When NaF or MFP pastes or solutions are employed, fluorapatite is the sole reaction product found in the tooth after 20 hours (CaF_2 sloughs off). The weight of enamel dissolved from the tooth is calculated from the weight of dissolved calcium (assuming that calcium comprises 37.4% of the enamel composition by weight), and hence the equation used to compute enamel fluoride content is:

$$\text{ppm F} = \frac{\text{Weight of fluoride}}{2.67 \times \text{weight of calcium}} \times 10^6$$

No phosphorous determinations are required for this computation.

G. Calculation of fluoride uptake

To determine the amount of fluoride taken up by the enamel, subtract the pre-treatment enamel fluoride concentration (ppm F) from the post-treatment concentration (ppm F).

REFERENCES

1. McCann, H. G., "Determination of Fluoride in Mineralized Tissues Using the Fluoride Ion Electrode," Arch Oral Biol, 13:475-477 (1968).

2. Stearns, R. I., "Potential Errors in Analyzing Enamel for Fluoride Concentrations and Rate of Acid Dissolution Subsequent to Stannous Fluoride Treatments," J. Dent Res, 51(3): 747-755 (1972).

APPENDIX A

ANALYTICAL METHODS FOR PHOSPHOROUS, CALCIUM AND FLUORIDE IN TOOTH ETCH SOLUTION

I. Analytical Method for Phosphorous

Apparatus (for Phosphorus Determination)

Technicon AutoAnalyzer, including following modules and accessories, Technicon Corporation, Tarrytown, New York 10591.

Sampler II, part No. 127-A000.
Proportioning pump I, single speed, part No. 105-A-200-01.
Heating bath, adjustable 0-100°C with single coil 40 ft. x 1.6 mm ID, part No. 105-A102-01.
Colorimeter with 50-mm tubular flow cell, part No. 112-A000-02.
Recorder, standard, 1 pen, part No. 011-A000-01.
Filters, S&G narrow band, 660-18-28.
Set of apertures.
Sampler cam 20-2/1, part No. 127-1049.
Sample cups, 2-ml, conical or flat bottom.
Manifold constructed as per Figure 1.
Technicon accessories such as pump lines, tubing, nipples, etc., to construct manifold.
Recorder chart paper having height in absorbance units.

Reagents (for Phosphorus Determination)

Deionized distilled water - use for all reagent preparation.
Ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4\ H_2O$, such as Baker's Analyzed Reagent grade.
Alkylbenzene sulfonic acid, aqueous solution, 0.01M.
Sulfuric acid, concentrated, Analytical Reagent grade.
Ammonium molybdate in 2.5N H_2SO_4, wetted. To about 500 ml of water in a 1-liter volumetric flask carefully add with mixing 70 ml of sulfuric acid. Add 12.5 g of ammonium molybdate and dissolve. Add 10 ml of 0.01M alkylbenzene sulfonic acid solution from a graduate and fill to mark with water. Mix well.
Hydrazine sulfate, Eastman Organic Chemical, No. 575.
Hydrazine sulfate solution, wetted. Dissolve 0.5 g of hydrazine sulfate in about 500 ml of water in a 1-liter volumetric flask. Add 10 ml of 0.01M alkylbenzene sulfonic acid solution, mix, and fill to the mark with water. Mix well.
Wash water, deionized distilled water. Note: DO NOT WET with alkylbenzene sulfonic acid.
Standard Solution A. Dissolve 3.3950 g (after drying 2 hours at 105°C) of dibasic calcium phosphate, Analytical Reagent grade, in water in a 1-liter volumetric flask. Add 15 ml of 1:1 HCl, fill to the mark, and mix well. To be used as Standard for both Ca and P. Contains 1000 ug of Ca per ml and 772.8 ug of P per ml.

Standard Solution B. Add 32.35 ml of Standard Solution A from a 50-ml buret to a 500-ml volumetric flask. Fill to the mark with water and mix. Contains 64.70 ug of Ca per ml and 50 ug of P per ml.

Daily phosphorous standards: Pipet the following aliquots of Solution B into 100-ml volumetric flasks.

Ml Std. Solution B	Phosphorus Content ug/ml
0	0.0
1	0.5
2	1.0
4	2.0
6	3.0
8	4.0
10	5.0

Fill to the mark with deionized water and mix well.

Preparation of Tooth Etch Samples

Pipet a 1.0 ml aliquot of the tooth etch sample (or blank sample) into a 50-ml volumetric flask. Fill to the mark with deionized water and mix.

Procedure for Phosphorous Determination

Using the daily phosphorous standards and etch samples prepared above, fill AutoAnalyzer cups and load turntable as follows: standards in order of increasing concentration, followed by samples. After every ten samples, re-run one of the phosphorous standards.

The AutoAnalyzer is operated following the normal operating procedures recommended by the Technicon Corporation.

Calculation

Construct the baseline under all peaks. Note and record in absorbance units the height of each peak above the baseline. (Peak abs.-baseline abs.=Corrected peak height).

Prepare a standard curve by plotting the corrected peak heights of the standards vs. their phosphorus concentrations in ug/ml.

Using the corrected sample peak height and the standard curve determine the phosphorus concentration in ug/ml for each sample.

$$(ug/ml) \times 50 = \text{ug of phosphorus in the 1.0-ml aliquot}$$

This value is then multiplied by the total etch solution volume (15.0 ml) in order to determine the weight of phosphorus removed from the tooth.

Precision

Statistical analysis has shown that the 95% confidence limit of a single determination varies in a smooth fashion from ±2% relative at 5 ppm P to as much as ±20% at 0.5 ppm P.

II. Analytical Method for Calcium

Principle

The calcium content of the dissolved sample is determined by comparison to standard solutions using an atomic absorption spectrophotometer.

Apparatus

Atomic absorption spectrophotometer, such as Perkin Elmer Model 303, complete with calcium hollow cathode tube.
Pipet, graduated, 1-ml.

Reagents

Standard Solution A (refer to Method for Phosphorous). Dilute Calcium Standard Solution. Dilute 25 ml of Solution A from a pipet to 500 ml in a volumetric flask to prepare an intermediate solution. Dilute 25 ml of the intermediate solution from a pipet to 100 ml in a volumetric flask. Contains 12.5 ug of Ca per ml.
Lanthanum Oxide, Matheson Coleman and Bell.
Stock Lanthanum solution. Add 58.65 g of lanthanum oxide and about 200 ml of water to a 1-liter volumetric flask. Add slowly, while mixing, 250 ml of conc. HCl. Fill to the mark and mix well.

Procedure

Pipet a 1.0 ml aliquot of the tooth etch (or blank) sample into a 25-ml volumetric flask. Add 5.0 ml of lanthanum solution and dilute to the mark with deionized water. Mix well

Prepare a set of standards by transferring to 6 appropriately numbered 25-ml volumetric flasks 0, 1, 2, 3, 4, and 5 ml of the dilute standard calcium solution (12.5 ug of Ca per ml) by pipet. Add 5 ml of lanthanum solution. Dilute to volume with deionized water and mix well. These standard solutions contain 0, 0.5, 1.0, 1.5, 2.0, and 2.5 ug of Ca per ml, respectively.

Calcium response is influenced by slight parameter changes. If the aspiration rate changes, or the fuel pressure drops, or the burner starts to build up a salt residue, the null response will be lowered. To detect any change, the standards and samples are read alternately. Thus the first set of 6 samples are sandwiched between the 6 standards used to plot the calcium curve; that is, a standard is read first, a sample second, and so on, but the standards are read in a random manner, not in order of magnitude. The next 6 samples are read in similar fashion. If a definite drop occurs for a standard, continue the set (6 standards - 6 samples). If the following standard values are not changed then reread the low standard. If the change occurs for the following standards then the burner must be shut down and cleaned.

Reset parameters to produce original calcium response and then continue. The 1971 Revision of Analytical Methods for Atomic Absorption Spectrophotometry published by Perkin-Elmer and the Instructions - Model 303 Atomic Absorption Spectrophotometer 303-0001 are to be used as references. Instrument parameters are as follows:

Nitrous Oxide	40# tank gage, 30# Burner Regulator, 4.6 flow
Acetylene	10# tank gage, 10.8 flow, change tank at 150#
Wavelength Reading	211.4 Visible
Cathode	Calcium 20 ma.
Burner	Perkin-Elmer Nitrous Oxide 2" slit. (5/8" orange cone)
Gain	5.4
Slit	3
Meter Response	2
Scale	x2
Aspiration rate	2.5 - 3.0 ml/min with one minute rinse between readings.

Calculations

An average null response is obtained for each of the 6 standards in percent absorption and is converted to absorbance through the use of a convenient conversion table contained in the Perkin-Elmer's 1971 Revision of Analytical Methods, Figure 4. As lanthanum oxide has trace calcium the "0" or reagent blank will have a positive absorbance and is subracted from both standards and samples. The average standard curve is plotted in ug Ca/ml vs. absorbance and the corrected samples compared. The general formula for calculation is:

(ug Ca/ml) x 25 ml = ug Ca in the 1.0-ml aliquot

The value obtained for each sample is then multiplied by the total etch solution volume (15.0 ml) to give the weight of calcium etched from the tooth.

III. Analytical Method for Fluoride

Principle

Etching solutions of tooth enamel are dilute perchloric acid solutions, buffered by trisodium citrate. The activity of fluoride in an etching solution is measured by means of an Orion fluoride specific ion electrode, and the fluoride concentration is read from a standard curve prepared with known amounts of fluoride.

The use of trisodium citrate serves to buffer the solution at a pH of about 5.6 and eliminates interference from calcium, phosphate, and aluminum.

Apparatus

pH Meter, such as Corning Digital 112 Research pH Meter or other meter capable of reading to ± 0.1 millivolt.

Fluoride specific ion electrode, Orion No. 94-09A, from Orion Research Incorporated, 11 Blackstone St., Cambridge, Mass. 02139.

Reference electrode, Orion No. 90-01, with Orion filling solution No. 90-00-01.
Magnetic stirrer, such as Corning Model No. PC 351. This stirrer is recommended because it runs cool even with prolonged stirring periods.
Magnetic stirring bars, 10 mm by 3 mm, Teflon-coated, such as Cat. No. 8546 from Cole-Parmer Instrument Co., 7425 North Oak Park Avenue, Chicago, Illinois 60648.
Hollow stoppers, polyethylene, size No. 10, Nalgene Cat. No. 6190.
Polyethylene bottles, 500-ml, 1000-ml.
Graduated cylinder, 50-ml.
Volumetric flasks, 250-ml, 500-ml, 1000-ml.
Pipets, 10-ml, 20-ml, 50-ml, 100-ml.

Reagents

Perchloric acid, 70% Reagent grade.
Perchloric acid, 0.50M. Measure 43 ml of 70% perchloric acid from a graduate into a 1000-ml volumetric flask. Make up to volume with distilled water and mix well. Standardize the acid by titrating 35 to 40 ml with standardized 0.5N NaOH as in A.M. 0.030, Procedure A. Adjust with water or acid until the molarity of the acid is between 0.495 and 0.505.
Trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), Reagent grade, Mallinckrodt No. 0754.
Trisodium citrate solution, 1.0M. Dissolve 294.1 grams of trisodium citrate dihydrate crystals in distilled water and dilute to volume in a 1000-ml volumetric flask with additional water, and mix. Store in a polyethylene bottle.
Sodium fluoride, Reagent grade.
Sodium fluoride solution, 2500 ppm fluoride. Dissolve 1.3816 grams of sodium fluoride in distilled water, make up to volume in a 250-ml volumetric flask with additional water, and mix well. Store in a polyethylene bottle.
Sodium fluoride solution, 50 ppm fluoride. Accurately pipet 20 ml of the 2500 ppm fluoride solution into a 1000-ml volumetric flask. Dilute to volume with distilled water and mix well. Store in a polyethylene bottle.
Sodium fluoride solution, 0.5 ppm fluoride. Accurately pipet 10 ml of the 50 ppm fluoride solution into a 1000-ml volumetric flask. Dilute to volume with distilled water and mix well. Store in a polyethylene bottle.
Sodium fluoride standard solutions for standard curve. Prepare a series of standard solutions 0.1M in perchloric acid and 0.4M in trisodium citrate, containing 0.05, 0.10, 0.15, 0.20, 1.0, 2.0, 10.0, and 20 ppm fluoride, as follows:
Pipet 100 ml of 0.5M perchloric acid and 200 ml of 1.0M trisodium citrate solution into each of eight 500-ml volumetric flasks. Pipet into each flask an appropriate volume of either 0.5 ppm or 50.0 ppm fluoride solution according to the following table.

Ml of 0.5 ppm F soln per 500 ml	Ml of 50 ppm F soln per 500 ml	ppm F in standard soln
50	-	0.05
100	-	0.10
150	-	0.15
200	-	0.20
-	10	1.0
-	20	2.0
-	100	10.0
-	200	20.0

Dilute each solution to volume with distilled water, mix well, and transfer to polyethylene bottles.

Fluoride Electrode Operation

Visually check the fluoride electrode to make sure the crystalline tip is not cracked. Connect the lead of the electrode to the shielded input jack of the pH meter.

Turn the meter onto STANDBY function and place the electrodes in distilled water for about 30 minutes to allow them to equilibrate.

The detailed instructions supplied with the pH meter and the Orion electrodes should be familiar to the operator.

Preparation of Standard Curve

Pour about 5 ml (exact volume not critical) of the lowest ppm fluoride standard solution into a hollow No. 10 polyethylene stopper. Place the stopper on the magnetic stirrer. If the Corning stirrer is not available, it will be necessary to insulate the stopper from a stirrer with a piece of plastic foam so that the solution will not be warmed. Insert the electrodes in the solution and drop the small stirring bar between them. Stir at a moderate rate.

Adjust the meter to read millivolts. The fluoride electrode does not always respond rapidly, and 15 to 20 minutes may be required to obtain stable readings. Observe the millivolts produced. Record the reading and its algebraic sign when a reading stable within ± 0.1 mv is obtained over a 30-second interval. The solution must be stirred during the reading. Remove the electrodes, rinse with distilled water, and dry with a soft paper tissue.

Determine the millivolt readings on the other standard solutions in a similar manner. Proceed in order from the solution with the lowest ppm fluoride concentration to the highest. A new standard curve must be obtained with each set of samples.

Plot the millivolts against the corresponding ppm of fluoride on semi-logarithmic paper, using the log scale for the ppm of fluoride. The entire range of standards will require four-cycle semi-log paper. A typical

example of such a curve is attached as Figure 2 for illustrative purposes only. In practice, it is entirely possible that the ppm of fluoride in etching solutions may lie within a single decade. In such instances, only these standards covering the range need to be used. For example, if the concentrations of fluoride in a set of etching solutions are between 0.2 and 2.0 ppm of fluoride, only a three-point plot as shown in Figure 2 would be required.

Should an unknown fall outside the plotted decade, two-cycle semi-log paper can be used and the plot extra polated into the next decade. Only one additional point in this range need be determined to establish the validity of the curve. Below 0.1 ppm the standard curve is not a straight line and a multipoint curve is needed. Values below 0.02 ppm can only be estimated, as this range is outside the lower limit of sensitivity of the electrode.

Procedure

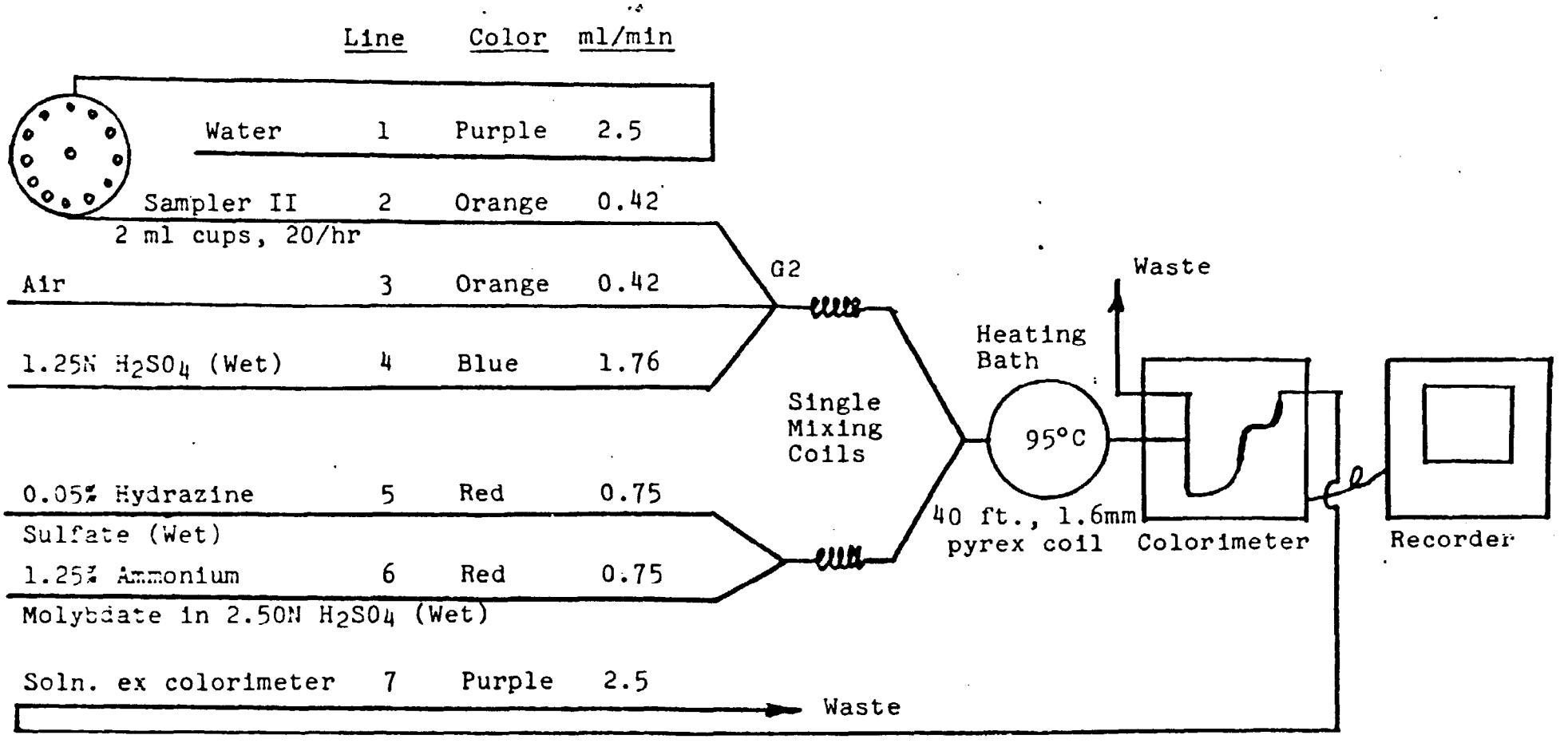
Samples must be 0.10M in perchloric acid and 0.4M in citrate.

Pour about 5 ml of the sample etching solution into a hollow No. 10 polyethylene stopper. Place the stopper on a magnetic stirrer that will not warm up, insert the electrodes, and add a magnetic stirring bar. Read the potential in millivolts, following the procedure used for the standard solutions.

Determine the level of fluoride in the sample solution by referring to the standard curve. Report the fluoride to the nearest 0.01 ppm.

FIGURE 1

Phosphate AutoAnalyzer Schematic



Notes:

1. All solutions except sample and water are "wetted" with 10 ml .01M alkylbenzene sulfonic acid per liter.
2. Full scale response 12.5 ppm P_2O_5.
3. Colorimeter filters 660-18-28, aperature #1.

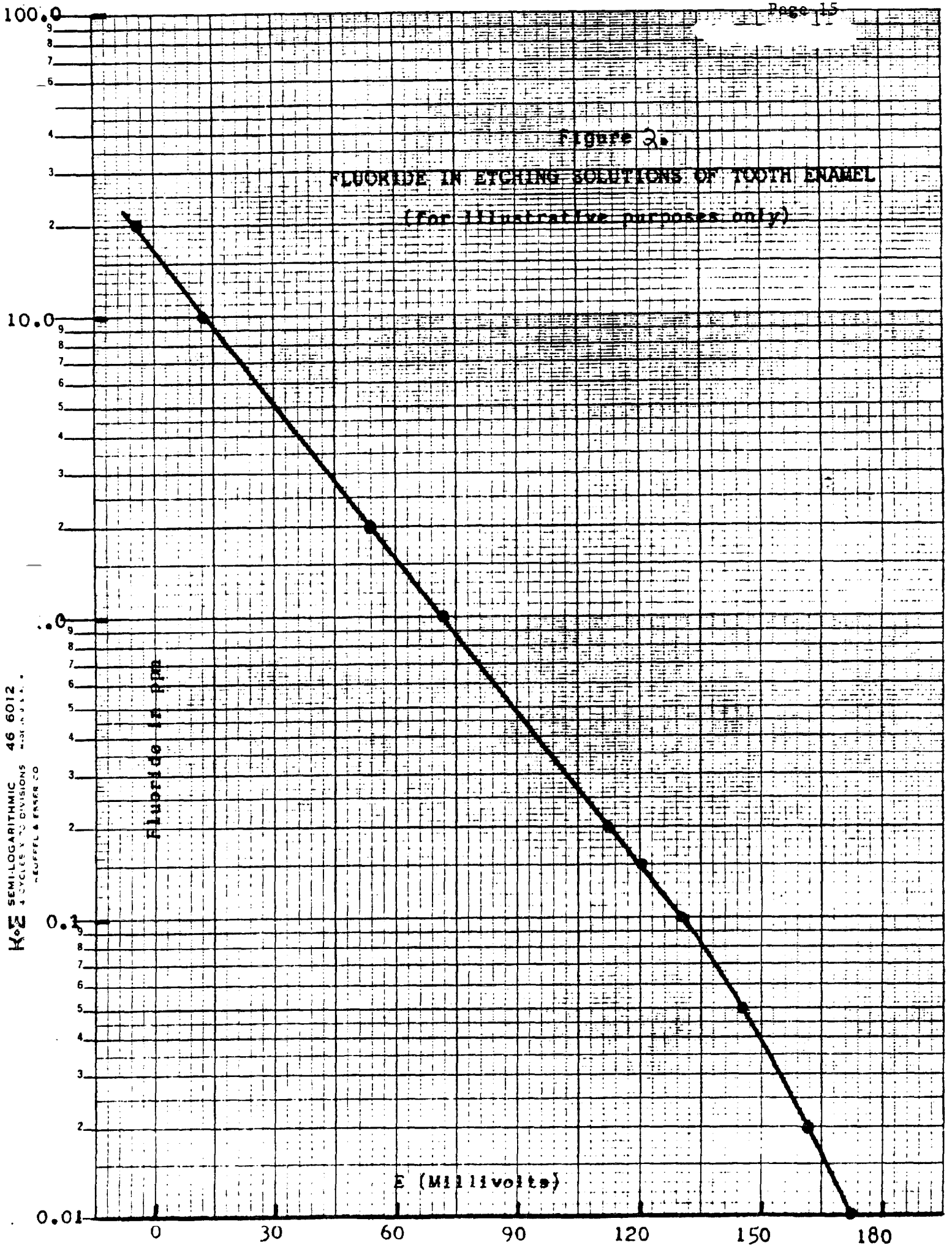
Reference:

AOCS Official Method Ca 12-55.

Figure 2.

FLUORIDE IN ETCHING SOLUTIONS OF TOOTH ENAMEL

(for illustrative purposes only)



STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #43

TITLE: DETERMINATION OF FLUORIDE UPTAKE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

f. Sodium Monofluorophosphate/Calcium Carbonate

TEST METHOD #43

DETERMINATION OF FLUORIDE UPTAKE

This method compares the fluoride content of human dental enamel powder which was treated with a dentifrice to that of enamel powder which was treated with a water control.

Treatment Stages

A. Weigh out (using regular weighing paper) 200 ± 1 mg of enamel (100-200 mesh powdered human dental enamel) for each cream being tested and transfer to 400 ml beakers. Include an enamel sample for water control.

B. Next, prepare the test solutions. Thirty gram dental cream - ninety gram distilled water slurries are prepared in 250 ml. Erlenmeyer flasks and agitated for thirty minutes (by a wrist-action shaker - Burrell Corp. Model BB - #10 speed). The slurries are then transferred to centrifuge tubes and centrifuged for twenty minutes using a Servall super speed angle centrifuge run at approximately twenty-three thousand RPM (powerstat set at 100).

C. Decant the supernatant solutions into 150 ml beakers and adjust the pH to 6.6 ± 0.2 by the dropwise addition of 1.0 molar acetic acid or NaOH.

D. As soon as possible, add seventy-five ml portions of the test solutions (measured in 100 ml graduates) to the enamel samples and seventy-five ml of distilled water to the control sample. Stagger the start of the reactions by one minute intervals.

E. Agitate the treatment systems by swirling (using a wrist-action) for about one-half minute at fifteen minute intervals starting with the actual addition of the test solution to the enamel. Treat the enamel samples for exactly one hour. Thirty seconds prior to the end of the hour, swirl the enamel again, this time tilting the beaker so that the enamel collects at the wall-floor junction.

F. At the end of the treatment time, decant the supernatant solution through a 200 mesh screen (U.S. Standard Sieve Series) and wash the enamel sample onto the screen with the aid of a wash bottle. Each screen is supported by a 5.75" funnel on top of a one liter Erlenmeyer flask. Immediately wash the enamel samples with one liter of distilled water to terminate the treatment.

G. Dry the treated enamel (on the screens) overnight at 37°C.

Fluoride Analysis

A. Remove the treated enamel samples from the oven and allow them to cool for one half hour.

B. Weigh out about 0.1 g of each enamel sample and transfer to a 25 ml volumetric flash. Add 0.5M $HClO_4$ to mark and swirl in order to dissolve the powder.

C. Remove 0.5 ml of the acid solution and add to 0.5 ml of modified total ionic strength buffer (TISAB*, adjusted with NaOH) so that the final pH of the mixture is 5.2. The fluoride content is measured with a fluoride ion specific electrode.*

D. Calculation of fluoride uptake by enamel

$$\text{F in enamel (ppm)} = \frac{\text{Electrode reading (ppm)} \times 50}{\text{Sample wt (g)}}$$

F uptake (ppm) = F in dentifrice-treated enamel (ppm) -

F in water-treated enamel (ppm)

*Orion Research, Cambridge, Mass.

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #42

DETERMINATION OF THE FLUORIDE UPTAKE FROM A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #42

DETERMINATION OF THE FLUORIDE UPTAKE FROM A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the amount of fluoridated hydroxyapatite formed when a powdered hydroxyapatite sample is treated with a dentifrice extract. This procedure is based upon a method described by Duke and Forward in Caries Research 12,12,1978.

Reagents	Suggested Type or Source
KOH	Baker Analyzed reagent
NaF	Baker Analyzed reagent
Methanol, Absolute	Baker Analyzed reagent
Lactic Acid	Baker Analyzed reagent
Hydroxyapatite	Baker Analyzed reagent
TISAB Solution	Orion Research Inc., Mass. 02139
Deionized Water	

Synthetic Saliva: The synthetic saliva used in this test is prepared by diluting 0.23 gm of H_3PO_4, 1.5 gm of KCl, 1.0 gm of $NaHCO_3$ and 0.22 gm $CaCl_2$ with 500 ml of deionized water. Transfer this solution to a 1000 ml volumetric flask and dilute to 1000 ml with deionized water.

Potassium lactate buffer:
Weigh 650 gm of KOH (AR) and dissolve in 10 liters of deionized water. Add, with constant agitation, 1.1 liters of concentrate lactic acid until complete solution. Add then enough quantity of deionized water (approx. 8.9 liters) to complete 20 liters. The fluid pH of the solution is 4.7 $\pm$ 0.1.

Apparatus	Suggested Type or Source
Orion Model 701 Ionalizer	Orion Research Inc.
Fluoride Specific Ion Electrode	Orion Research Inc.
Centrifuge, High speed	Clinical Model IEC Inc.
Plastic Disposable Syringes	B-D 50 ml
Magnetic Stirrer	Labline "Magna-Stir"

Apparatus (continued)	Suggested Type or Source
Stirring bars	S.G.A.
Polyethylene beakers and flasks	S.G.A.
Volumetric flasks	S.G.A.
Filter paper	Whatman No. 541
Membrane Holder	Millipore Inc.
Filter cone	Millipore Inc.

Procedure

1) Weigh 20 gm ± 1.0 gm of dentifrice in a 50 ml polyethylene beaker and slurry with 20 ml of synthetic saliva.

2) Transfer the slurry to a 50 ml centrifuge tube and centrifuge at 3000 rpm for 10 min.

3) Transfer the supernatant liquid to a filter paper cone supported by a plastic base into another centrifuge tube (50 ml capacity) and centrifuge for 45 min. at 1800 rpm.

4) Take 5 ml of filtrate per sample, transfer to a 25 ml volumetric flask and complete up to volume with synthetic saliva.

5) 30 - 40 mg of hydroxyapatite are added to the 25 ml fluoride solution and magnetically stirred for 1 min. at room temperature.

6) The suspension is filtered using Whatman No. 541 filter paper held in a Swannex-25 membrane holder attached to a disposable plastic syringe.

7) The powder held on this filter is washed twice with 20 ml deionized water and once with 20 ml methanol.

8) The powder is dried to constant weight for 24 hours at 45°C.

9) The powder is divided into two parts: sample A = 1/3 and sample B = 2/3 and each reweighed.

10) Sample A is dissolved in 25 ml 1 M potassium lactate buffer in a plastic container. The ionic fluoride content of the resultant solution is measured directly using an Orion specific electrode calibrated with appropriate standards.

11) The total uptake of fluoride by the hydroxyapatite is calculated.

12) Sample B (2/3) is equilibrated overnight with 50 ml 1 M potassium hydroxide in a closed plastic container on an orbital shaker.

13) The suspension is centrifuged for 20 min. at 2500 rpm and the supernatant decanted.

14) Take 25 ml of supernatant liquid and transfer to a plastic beaker. Add 2 ml of concentrated lactic acid to bring the pH to 5 - 7. Cap and let stand for 24 hrs.

15) The ionic fluoride is then determined by means of the specific ion electrode and appropriate standards.

16) This represents the amount of fluoride deposited on the surface of the hydroxyapatite powder in the form of calcium fluoride.

17) The residue from centrifugation in Step #13 is slurried with 25 ml of 1 M KOH.

18) The resultant solution is filtered using Whatman No. 541 filter paper held in a Swinnex-25 membrane holder (Millipore) attached to a disposable plastic syringe.

19) The powder held on this filter is washed with 20 ml 1 M KOH, 20 ml deionized water and 20 ml methanol.

20) The residue is dried to constant weight, and then dissolved in 25 ml potassium lactate buffer.

21) The resultant solution is analyzed for ionic fluoride as before. The concentration of fluoride ion present as fluoridated hydroxyapatite is then calculated.

22) To determine the free fluoride, weigh about 2.0 gm $\pm$ 0.1 gm of dentifrice and slurry with 6 gm of synthetic saliva.

23) Transfer the slurry to a filter paper cone supported by a plastic base into a centrifuge tube (50 ml capacity) and centrifuge at 1600 rpm for 10 min.

24) Take 1 ml of the filtrate and dilute to 10% of its original concentration with 1 ml of TISAB and 8 ml of deionized water. This solution is analyzed for ionic free fluoride as before.

Calculation

$$\text{FHA (approximate)} = \text{Total } F^- - CaF_2$$

$$\text{FHA} = \frac{\text{Reading FHA in ppm}}{\log F^-}$$

$$\log F^- = \log \left(\frac{100}{\text{(ppm Free } F^-)} \right)$$

TEST METHOD #17

Determination of Soluble Tin (II) in Stannous Fluoride-Calcium Pyrophosphate Toothpaste by The Oiodate Titration Method

Scope

This method is an empirical one, designed for the determination of soluble, stannous tin in stannous fluoride toothpastes after 1 min. brushing times and normal dilutions. The time and slurry and the time until centrifuging begins are critical and must be rigidly adhered to if reproducibility is to be attained. The standard deviation (B. A. D.) for the method is 8.8 ppm at the 200-500 ppm level.

Reagents

Oxygen-free distilled water	Bubble nitrogen through distilled water for at least 30 minutes.
Hydrochloric Acid	Reagent ACS grade
Potassium Iodate	ACS grade
Nitrogen Gas	Regular (0.05% O_2 maximum)
Potassium Iodide Solution	10% w/v

Apparatus

Centrifuge, high speed	International Model CS, UV, 2N or 2 EXD with high speed attachment
Centrifuge tubes	25 ml International No. 298
Stirring rods	8-inch, with a 2-inch piece of tygon slipped over one end, such that an inch of tubing extends beyond the rod. The extended tubing is sliced to form 4 fingers.
Magnetic stirrer	Mag- Mix # 65904 or equivalent
Magnetic stirring bar	Teflon covered.
Nitrogen Atmosphere Chamber	A large stainless steel beaker with cover, constantly purged with nitrogen.
Micro buret	10 ml, graduated by 0.05 ml
Pipettes	2, 5, and 10 ml
Dispensing flask	5 ml delivery
Beakers	100 ml, 250 ml
pH Meter	Any commercial model, expanded scale preferred.
Electrode	Beckman Platinium 1273
Electrode	Beckman Fiber Junction Calomel.
Rubber Stopper	No. 13, with 5 holes to accomodate the two electrodes, the buret tip, and nitrogen inlet and outlet tubes.

Volumetric flask 1000 ml

Clock-timer

Preparation of KIO_3 Solution - 0.005 N

Weigh 0.1784 ± 0.0001 gram potassium iodate, add 200 ml oxygen-free distilled water and stir until completely dissolved. Dilute to 1 liter with oxygen-free distilled water in a volumetric flask and mix well. (If a standardized, 0.1N solution of potassium iodate is available, this may be diluted to 0.005N by pipetting 50 ml of the 0.1N solution into a 1-liter volumetric flask and making to volume with oxygen-free distilled water.)

Preparation of Supernatants

Two samples can conveniently be prepared at the same time. Discard the first inch of toothpaste from the tube, then weigh 10.0 grams of each paste into 100 ml beakers, using a Harvard Trip or similar balance. Pipette 30 ml of oxygen-free distilled water into each of the beakers and then set a clock-timer for 5 minutes before starting the slurrying. Using tygon-tipped stirring rods, slurry back and forth between the samples (10-15 seconds in each sample) for a total of 2 minutes. Pour 20-22 ml of the uniform slurries into centrifuge cups, taking care to balance the two cups very precisely. Five minutes after starting to slurry the pastes (by the clock-timer), start the centrifuge and spin the slurries for 30 minutes at 11,000 r.p.m. Two other pastes may be started at this time and the centrifuge may be stopped to add these two samples at the end of their 5-minute time period. All four samples are then centrifuged for 30 minutes. After centrifuging, the clear supernatant should be carefully decanted into a labeled vial and placed in a nitrogen atmosphere chamber until titrated. This solution should be titrated within 20 minutes for stannous tin. The same supernatant can be used for the soluble fluoride determination and is stable for at least one week.

Operation

Add into a 250-ml beaker about 125 ml of oxygen free distilled water, 3 ml KI solution, 5 ml of conc. HCl and a stirring bar. The HCl and KI can be added from dispensing flasks. Pipette 2.0 ml of the prepared supernatant (if the Sn^{++} level is $>$ 350 ppm) into the diluted HCl solution. (If the Sn^{++} level is expected to be $<$350 ppm, a 5.0 or 10.0 ml aliquot will be needed -- 5.0 ml for 150-350 ppm, and 10.0 ml for $<$150 ppm.) Immediately place the beaker on a magnetic stirrer and cover with the rubber stopper containing the two electrodes, buret, and nitrogen inlet and outlet tubes. Start the nitrogen flow and the mixer, making sure that the electrodes are in contact with the solution.

Set the pH meter on the millivolt scale and start the titration. Titrate slowly, carefully observing the pH meter needle. The largest change in millivolts for a single drop of KIO_3 added is the endpoint. The deflection is very large and easier to detect if an expanded-scale pH meter is used. Record the volume of titrant used.

Calculations

$$\text{ppm } Sn^{++} = \frac{\text{ml } KIO_3 \times \text{N } KIO_3 \times 0.0594 \times 10^6}{\text{ml supernatant titrated}}$$

Note: If the KIO_3 is exactly 0.0050N, the calculations can be reduced to:

ppm Sn^{++} = Titration x 148.5 for 2 ml aliquot

ppm Sn^{++} = Titration x 59.4 for 5 ml aliquot

ppm Sn^{++} = Titration x 29.7 for 10 ml aliquot

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #18

DETERMINATION OF SOLUBLE STANNOUS ION

Recommended for the following systems:

a. Stannous fluoride and silica abrasive.

TEST METHOD #18

DETERMINATION OF SOLUBLE STANNOUS ION

Principle

The "available" tin is that portion of the total tin that is found in the supernatant liquid after centrifuging an aqueous slurry of toothpaste under prescribed conditions. That portion of the "available" tin present in the stannous state is determined by titration with standard iodine solution.

Apparatus

B-18 Flask. A side arm and a delivery tube, the latter reaching to within 2 to 5 mm of the bottom of the flask, are sealed into a 250-ml Erlenmeyer flask having a ℸ 24/40 outer joint (Corning No. 5000). See the attached Figure 1. The flask should be marked to indicate a volume of 60 ml. These flasks are not stock items, but can be made readily by any supply house glassblower.
Condenser tube, 60 cm long, with ℸ 24/40 inner joint to fit the B-18 flask, such as SGA Scientific Inc. Item No. JC-7050.
Centrifuge tubes, round bottom, polypropylene, 50-ml, such as SGA Scientific Inc. Item No. C-3512-2.
Centrifuge tube closures to fit 50-ml centrifuge tubes, such as SGA Scientific Inc. Item No. C-3512-4.
Burets, 10-ml, and 50-ml, the latter only having a large tip for rapid delivery.
Erlenmeyer flask, 50-ml.
Graduated cylinders, 50-ml.
Pipet, 10-ml.

Reagents

Carbon dioxide, cylinder with regulator.
Hydrochloric acid, concentrated, Reagent grade.
Iodine solution, about 0.01N, standard. Prepare and standardize daily.
Starch indicator solution.
Oxygen-free water. Boil distilled water for 5 minutes and cool under a blanket of CO_2 while bubbling the gas through the water.

Procedure

Care must be exercised throughout the determination to prevent the contact of air or oxygen with the sample. Maintain a blanket of CO_2 above the sample at all times.

Weigh 2.5 to 3.0 grams of paste, to an accuracy of 1 mg, into a centrifuge tube which has just been purged with CO_2.

Keep the centrifuge tube capped as much as possible during the entire extraction procedure.

Add from the 50 ml buret 1- to 2-ml increments of oxygen-free water, thoroughly mixing with the paste (use a glass rod) after each addition until a smooth slurry is obtained (about 10 ml of water). Continue the addition of water until the total volume added is 10 times the weight of paste taken. During this procedure, maintain a flow of CO_2 into the centrifuge tube by means of a glass U-tube hooked over the side.

Mix by stirring, rinse off the glass rod with 1 or 2 ml of oxygen-free water from the buret, cap the tube, and centrifuge at 5000 rpm for 15 minutes. Read the total volume of water added from the buret to the nearest 0.1 ml.

Immediately decant the supernatant liquid into a 50-ml Erlenmeyer flask, which has been purged with CO_2, and stopper.

Pipet a 10-ml aliquot of the solution in the Erlenmeyer flask into a B-18 flask containing 50 ml of distilled water and 25 ml of concentrated hydrochloric acid that have previously been boiled for about 5 minutes and cooled under a blanket of CO_2.

Titrate immediately with standard 0.01N iodine solution to the starch endpoint, maintaining a flow of CO_2.

Determine a blank by carrying a toothpaste of similar formulation, but without stannous fluoride, through the entire procedure.

Calculation

$$\frac{(V-B) \times N \times 5.935}{\frac{W \times 10}{M}} = \% \text{ "Available " Stannous Tin}$$

or

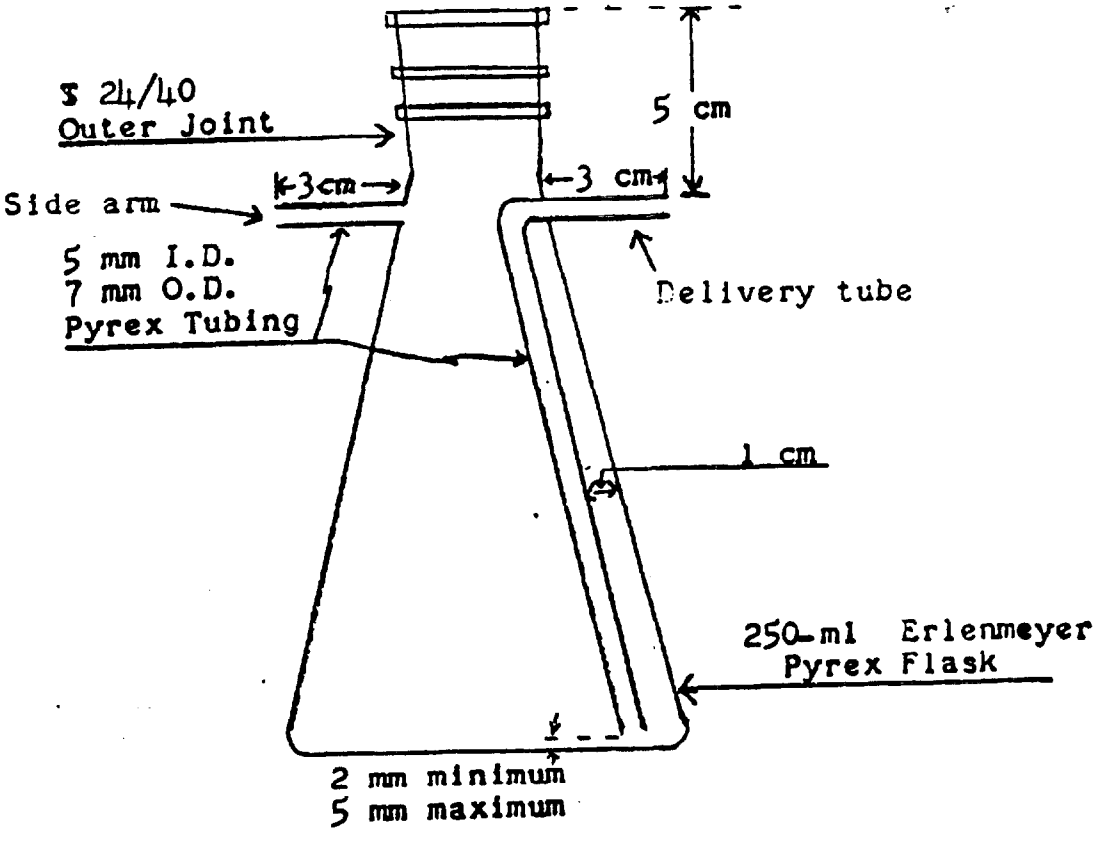
$$\frac{(V-B) \times N \times M \times 0.5935}{W} = \% \text{ "Available" Stannous Tin}$$

Where:

V = Ml of iodine solution required to titrate the sample.
B = Ml of iodine solution required to titrate the blank.
N = Normality of 0.01N iodine solution.
M = Ml of water used to prepare slurry.
W = Weight of sample in grams.

This calculation does not take into account the contribution of the water-soluble components of the paste to the final volume. Assuming the average content of water-soluble materials to be 45% of the paste, this effect may be compensated for by substituting (M + 0.45 W) for M in the calculation.

Figure 1. Titration Vessel



Exact dimensions not critical.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #19

TITLE: DETERMINATION OF SOLUBLE STANNOUS ION

Recommended for the following systems:

a. Stannous Fluoride/Insoluble Sodium Metaphosphate

TEST METHOD #19

DETERMINATION OF SOLUBLE STANNOUS ION

SCOPE

This method is applicable to the determination of soluble tin in dental creams.

PRINCIPLE

A water slurry of the dental cream is centrifuged. A portion of the supernatant is diluted and aspirated into the flame of an atomic absorption spectrophotometer.

APPARATUS REQUIRED

(1) Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer, or equivalent

(2) Tin hollow cathode lamp, P-E Model 303-6074 or electrodeless discharge lamp, P-E Model 303-6274

(3) Nitrous oxide burner head

(4) Acetylene gas cylinder with appropriate regulator

(5) Nitrous oxide cylinder with appropriate regulator

(6) Magnetic stirrer and stirring bars

(7) Suitable centrifuge capable of 3,000 rpm and 100-ml. tubes

(8) Polypropylene labware

REAGENTS REQUIRED

Standard tin solution (1,000 ug./ml.), Harleco or Fisher

PROCEDURE

Set up the instrument as per the manufacturer's instructions for tin using nitrous oxide-acetylene, a wavelength of 286 nm. and a slit of 0.7 nm.

(1) Pipet 5 and 10 ml. of the 1,000 ug./ml. tin standard into two separate 100-ml. polypropylene volumetric flasks. Dilute to volume with distilled water and mix well. (These are equivalent to 50 and 100 ug. Sn/ml.)

(2) Accurately weigh 20.00 ±1.00 gm. (to the nearest 0.1 gm.) of sample into a 100-ml. polypropylene beaker. Add 50 ml. of distilled water and a magnetic stirring bar. Stir 15 minutes.

(3) Transfer the slurry quantitatively with the use of distilled water into a 100-ml. polypropylene volumetric flask. Dilute to volume with distilled water and mix well.

(4) Transfer some of the slurried solution into two 100-ml. centrifuge tubes, balance and centrifuge at 3,000 rpm for 30 minutes.

(5) Pipet 10 ml. of one of the supernatants into a 50-ml. polypropylene volumetric flask. Dilute to volume with distilled water and mix well.

(6) Using distilled water as the blank and the two standard solutions standardize the instrument to read directly in concentration (ug./ml).

(7) Aspirate the sample solution into the flame and read its concentration in ug./ml. from the instrument.

CALCULATIONS:

$$\frac{\text{ug. Sn/ml. (Step 7)} \times 100 \times 5}{S} = \text{ug. Sn/gm.}$$

Where:

ug. Sn/ml. = Concentration read in Step 7
100 = Volume of solution
5 = Dilution factor
S = Sample weight in gm.

RT:ls

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #20

TITLE: DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^{=}$ ION

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #20

DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^=$ ION

SCOPE

This procedure is applicable to the determination of sodium monofluorophosphate (as F) in dental cream.

PRINCIPLE

The method is based upon the separation of the water soluble monofluorophosphate from the other soluble phosphates by column chromatography. All the phosphate species are exchanged on a strongly basic anionic ion exchange resin. Their differences of affinity for the resin permit their separation by ionic strength of an eluant such as KCl:

$$2\ R\cdot Cl + HPO_4 \text{ (orthophosphate)} \qquad R_2HPO_4 + 2\ Cl^-$$
$$2\ R\cdot Cl + PO_3F \text{ (monofluorophosphate)} \qquad R_2PO_3F + 2\ Cl^-$$
$$2\ R\cdot Cl + H_2P_2O_7 \text{ (pyrophosphate)} \qquad R_2H_2P_2O_7 + 2\ Cl^-$$

Where R represents the non-exchangeable insoluble polymeric portion of the resin.

The column is packed with a strongly basic anionic exchange resin and the various phosphate components are separated by elution. The eluate is continuously fed to an AutoAnalyzer unit which is assembled for automated phosphate analysis. After the individual phosphate species are hydrolyzed to the ortho form they are reacted to yield the molybdenum blue color. The intensity of color, which is proportional to the level of phosphate present, is determined by a colorimeter. The colorimeter response is fed to a recorder which gives a continuous trace of the individual phosphate composition of the sample stream. The monofluorophosphate level is calculated by relating the area under the MFP peak with that of a standard introduced, on the same column, ahead of the sample.

APPARATUS REQUIRED

Chromatographic Column, 10 mm O.D. 8 mm ID filled to a height of 12-14 cm with Dowex 1-X-8 or Biorad AG1-X-8 (200-400 Mesh) ion exchange resin. See Note A.

These columns are made by softening the middle section of a 60 cm length of 10 mm O.D. glass tubing in a flame and extending the ends to produce a constricted center portion. After cooling, the constriction is cut and the ends fire-polished. The constricted ends are fitted with 20 cm lengths of transmission tubing equipped with a N-8 nipple at one end. A small plug of glass wool is inserted into the constricted end and the column filled to the correct height with the ion exchange resin slurry in water. Another small plug of glass wool is inserted on top of the resin. Wash the resin with about 20 ml of 0.15 KCl to equilibrate it.

Technicon AutoAnalyzer unit, manufactured by Technicon Controls, Ardsley, N.Y. For this analysis the following AutoAnalyzer modules are required.

1. Sampler II using 8.5 ml sample cups and a 10 - 2/1 cam.
2. Proportioning Pump.
3. 95°C Heating Bath with two 40 foot, 2 mm ID glass coils.
4. Colorimeter with 15 mm flow cell and 660 mu filters.
5. Single Pen Recorder with optical density chart paper and a chart speed of 0.782 cm/min.

The modules with manifold are assembled according to Figure 1. This diagram is a suggested manifold which when used should give reproducible and accurate results. It may be necessary to diverge slightly. For example, if the liquid level in the ion exchange column tends to drop during analysis, the manifold line #2, orange-blue should be changed to a larger diameter tube. Chromatographic tubes which become aerated should be discarded and replaced with a fresh column.

REAGENTS REQUIRED

1. Sulfuric Acid 6N: To a 2-liter volumetric flask containing about 1,500 ml of deionized water add, very carefully, 333 ml of conc. C.P. sulfuric acid. Cool to room temperature, make to volume with deionized water and mix well. (Safety precautions should be observed when working with acid.)

2. Ammonium Molybdate: In a 2-liter volumetric flask containing 1,500 ml deionized water dissolve 30 gms of $(NH_4)_6MO_7O_{24}\cdot 4H_2O$ and add 200 ml of conc. C.P. sulfuric acid. Cool to room temperature, make to volume with deionized water and mix well. (Safety precautions should be observed when working with acid.)

3. Hydrazine Sulfate: In a 2-liter volumetric flask containing some deionized water dissolve 2 gms of $H_2NNH_2\cdot H_2SO_4$. Make up to volume with deionized water and mix well.

4. Stock Buffer Solution (pH 5.0): Weigh 70.0 grams of CH_3COOK and 18.0 grams of glacial acetic acid and dilute to 1000 ml with water. Adjust the pH to 5.0 by the additions of 2% acetic acid or KOH as necessary.

5. Potassium Chloride (0.15M): Weigh 22.5 grams of KCl, add 50 ml of stock buffer solution and dilute to 2000 ml with water.

6. Potassium Dihydrogen Phosphate (KH_2PO_4), Bakers' Analyzed Reagent Grade, Crystal, J. T. Baker Co. Catalog No. 3246, meets ACS specifications 99.0% purity minimum.

7. Standard P_2O_5 Solutions:

   a. P_2O_5 Stock Solution (1000 mg P_2O_5/1000 ml): Dry some KH_2PO_4 in an oven at 105°C for 1 to 2 hours. Accurately weigh 1.9180 gm to the nearest 0.1 mg and carefully transfer it to a 1-liter volumetric flask. Dilute to volume with deionized water after the salt is dissolved and mix well.

   b. 30 mg P_2O_5/500 ml - Standard: Accurately pipet 30.00 ml of the stock solution into a 500 ml volumetric flask. Dilute to volume with deionized water and mix well.

See Flow Diagram (Figure 1)

PROCEDURE

1. Start the proportioning pump and place reagent lines in reagents according to figure 1. Do not connect the column until step 2 is completed. (See Note B.) Turn on colorimeter and recorder.

2. Be sure 0.15M KCl is in the wash cup at the side of Sample II and that enough 0.15M KCl is in the head space of the column to leave 2 cm of liquid above the resin when the sample line is filled with liquid from the column. Connect the column to the sample line as shown in Figure 1.

3. While the instrument is being equilibrated and base line is being established, weigh a representative 4 gram sample to the nearest tenth milligram into a (100 ml) beaker.

4. Slurry with about 60 ml of distilled water and quantitatively transfer to a 100 ml volumetric flask. Dilute to volume and mix well. Be sure all small particles are broken up.

5. Centrifuge a portion of the diluted sample and filter the supernatant liquid into an 8.5 ml sample cup. Use Whatman #41 filter paper.

6. Place the sample cup with the sample from step 5 in the sample tray of Sample II according to the following order. (See Note C.) Use the 10-2/1 cam.

Tray Cup #1. 2N HCl
2. 0.15M KCl
3. 30 mg P_2O_5/500 ml standard (see Note C.)
4. 0.25M KCl
5. Sample
6. 0.15M KCl
7. 0.15M KCl
8. 0.15M KCl
9. 0.15M KCl
10. 0.15M KCl
11. 0.15M KCl
12. 0.15M KCl

After Cup #12 repeat Cup #1 thru #12 for continuous automatic analysis.

CALCULATIONS

Measure the area under the peak obtained for the phosphate standard and the monofluorophosphate peak as follows:

1. Measure the height of the peaks at the apex in absorbance units and deduct the absorbance reading due to the base line. This value is the corrected absorbance.

2. Divide the corrected absorbance by 2 and add the absorbance due to the base line. This value is the corrected half absorbance.

3. Using a millimeter rule, measure the width of the peak at the corrected half absorbance.

4. Calculate the area by multiplying the corrected absorbance (1) by the width at corrected half absorbance (3).

$$\frac{B \times 0.06 \times 2.03 \times 100}{A \times 5/100 \times 7.58} = \% \text{ Sodium Monofluorophosphate as F}$$

Where: A = Area of Standard in Absorbance/mm
B = Area of Sample in Absorbance/mm
S = Weight of original sample in milligrams (step 3) divided by 100
0.06/100 = mg P_2O_5 in aliquot of standard P_2O_5 solution (30 mg/500 ml)
2.030 = Factor to convert P_2O_5 to Monofluorophosphate
7.58 = Factor to convert Sodium Monofluorophosphate to F

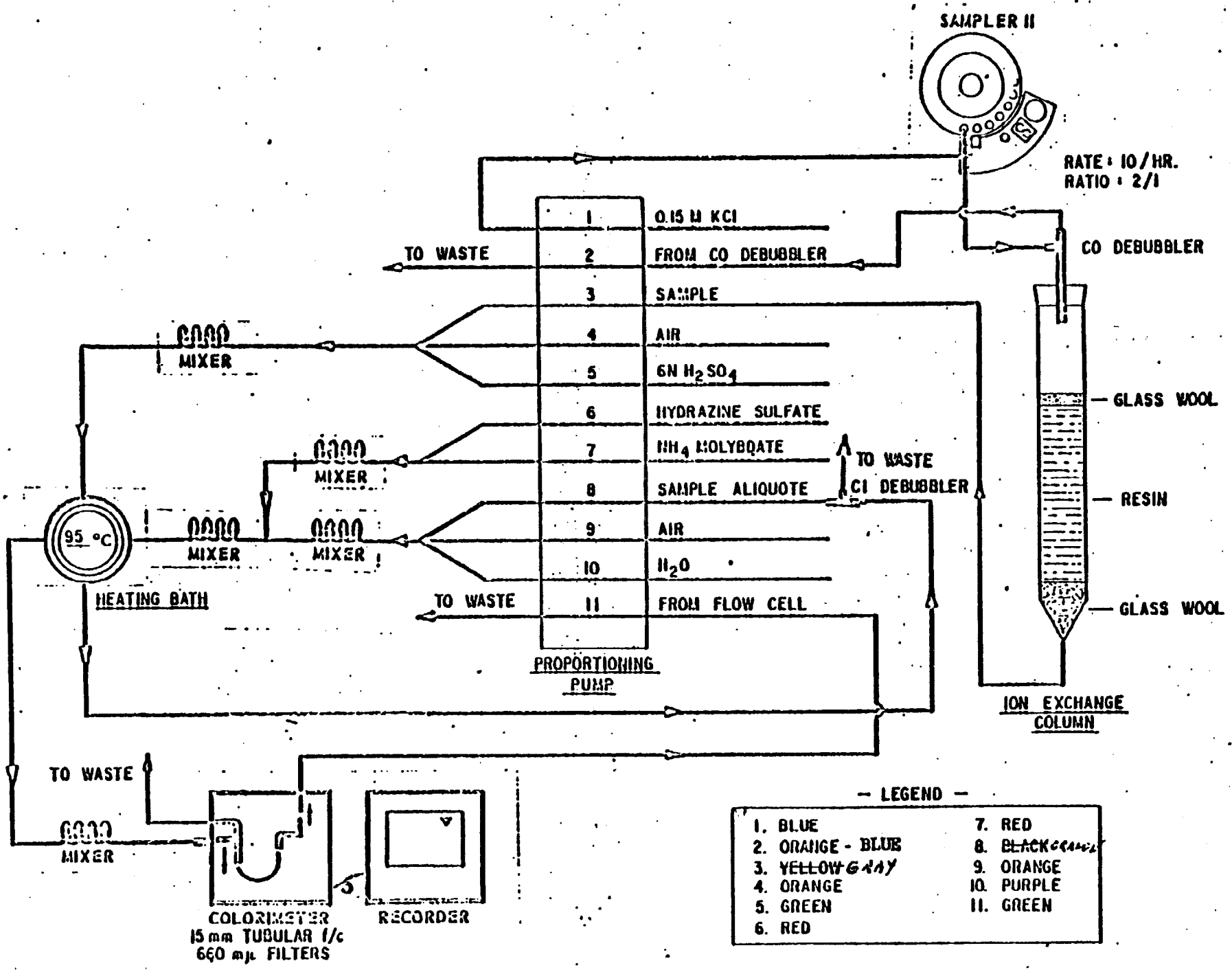
NOTES:

A. The resin is prepared by soaking it overnight in 2N HCl. After decanting the acid, wash well with water and 0.25M KCl. Store in water.

B. The CO debubbler module at the top of the column, see Figure 1, and the transmission tube from the column to the manifold sample line should be connected and disconnected at the beginning and end of each days operation. If this isn't done properly, the column may be pumped dry or otherwise become contaminated. Use a pinch clamp to close the sample line from the column when disconnected to prevent the column from draining dry.

C. At the beginning of each day the column should be conditioned by chromatographing two standard 30 mg P_2O_5 per 500 ml curves successively before samples are introduced. The second standard curve should be used for standardizing the first sample. Thus at the beginning of each days operation or the use of a new column the tray would be arranged as follows.

Tray Cup #1. 2N HCl
2. 0.15M KCl
3. 30.0 mg P_2O_5/500 ml
4. 0.15M KCl
5. 2N HCl
6. 0.15M KCl
7. 30.0 mg P_2O_5/500 ml
8. 0.15M KCl
9. 0.15M KCl
10. Sample
11. Continue with Cup #6 - 0.15M KCl


SAMPLER II
RATE : 10/HR.
RATIO : 2/1
1 0.15 M KCl
2 FROM CO DEBUBBLER
3 SAMPLE
4 AIR
5 6N H_2SO_4
6 HYDRAZINE SULFATE
7 NH_4 MOLYBDATE
8 SAMPLE ALIQUOT
9 AIR
10 H_2O
11 FROM FLOW CELL
TO WASTE
CO DEBUBBLER
MIXER
MIXER
MIXER
MIXER
95 °C
HEATING BATH
TO WASTE
TO WASTE
CI DEBUBBLER
GLASS WOOL
RESIN
GLASS WOOL
PROPORTIONING PUMP
ION EXCHANGE COLUMN
TO WASTE
MIXER
COLORIMETER
15 mm TUBULAR f/c
660 mμ FILTERS
RECORDER
— LEGEND —
1. BLUE
2. ORANGE - BLUE
3. YELLOW-GRAY
4. ORANGE
5. GREEN
6. RED
7. RED
8. BLACK-GRAY
9. ORANGE
10. PURPLE
11. GREEN

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #21

TITLE: DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^=$ ION

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #21

DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^{=}$ ION

PRINCIPLE

Determination of Sodium Monofluorophosphate is based upon the complete precipitation of $PO_3F^{=}$ by Ag^{+}. After removal of the soluble organic matter by filtration, the precipitate is ashed in the presence of Calcium Oxide to remove the gum. The fluoride is determined by the usual distillation procedure and calculated as Sodium Monofluorophosphate.

METHOD

APPARATUS REQUIRED

1. Steam Distillation Apparatus of borosilicate glass with band heater as shown in Figure I, LaPine Scientific Co., Irvington-On-Hudson, N. Y.
2. Glas-Col Heating Mantle, hemispherical to fit 2000 ml flask.
3. Variable Transformers (2) such as the "Powerstat" Type 116, Superior Electric Co., Bristol, Conn., or "Variac," Type V-5, General Radio Co., Cambridge, Mass. These transformers are available from most laboratory supply houses.
4. Centrifuge, capable of speeds up to 10,000 rpm.
5. Centrifuge tubes, Nickel, 1" x 4", (2).
6. Mechanical stirrer.
7. Nickel dish - 30 ml.
8. Muffle furnace.

REAGENTS REQUIRED

1. 80% Reagent Alcohol: To 200 ml of water add 800 ml of SD 3A Alcohol.
2. SD 3A Alcohol, Saturated with Ag_2PO_3F

   Preparation of Silver Monofluorophosphate (Ag_2PO_3F):

   A. Dissolve 0.2 gram of Sodium Monofluorophosphate in 10 ml of water.

B. Add 2 ml of 50% Silver Perchlorate solution and 50 ml of cold (0-5°C) SD 3A Alcohol. Mix well.

C. Filter the solution from step B through a Whatman No. 41 (or equivalent) filter paper and wash the residue twice with 25 ml portions of cold (0-5°C) SD 3A 80% Reagent Alcohol.

D. Quantitatively transfer the residue from step C to a 500 ml volumetric flask using cold (0-5°C) SD 3A Alcohol to complete the transfer. Dilute to volume with cold SD 3A Alcohol and shake vigorously for 5 minutes.

E. Allow the insoluble material to settle and store in refrigerator.

3. SD 3A Alcohol, 80%, Saturated with Ag_2PO_3F: Proceed as directed except that SD 3A 80% Reagent Alcohol is used in step D in place of SD 3A Alcohol.

4. Zirconyl Chloride Solution ($ZrOCl_2 \cdot 8H_2O$): Dissolve 0.265 gram of Zirconyl Chloride ($ZrOCl_2 \cdot 8H_2O$) in 50 ml of water. Add 700 ml of conc. HCl and dilute to volume with water at room temperature in a 1000 ml volumetric flask. Mix well.

5. Silver Perchlorate Solution: Dissolve 250 grams of anhydrous Silver Perchlorate ($AgClO_4$) in 400 ml of water and dilute to 500 ml.

6. Eriochrome Cyanine R Solution: Dissolve 1.8 grams of Eriochrome Cyanine R (obtainable from Geigy Chemical Corp., N. Y., N. Y.) in 100 ml of water and dilute to 1000 ml.

7. Phenolphthalein Indicator Solution.

8. Approx. 0.5N Alcoholic KOH Solution.

9. Calcium Oxide (CaO) (Fisher Scientific Co. Cat. #C-117).

10. Sodium Fluoride (NaF) - Reagent Grade.

11. Perchloric Acid ($HClO_4$) - 70%.

12. Hydrochloric Acid Solution: 7.00 ml of CHl (conc.) + 3.00 ml of H_2O.

13. Silver Nitrate Solution - 50% (aq.).

PROCEDURE

1. Accurately weigh 20 ± 0.2 grams (to the nearest 0.1 milligram) of sample into a 150 ml beaker. Add 60 ml of water and mix thoroughly with a stirring rod and then with a magnetic stirrer for 15 minutes.

2. Quantitatively transfer the dispersion to a 100 ml glass stoppered mixing cylinder using water to complete the transfer. Dilute to the 100 ml mark with water, stopper the cylinder and shake vigorously.

3. Transfer the dispersion from step 2 in equal amounts to 2 Nickel centrifuge tubes and centrifuge at 10,000 rpm for 20 minutes.

4. Pipet 10 ml of the clear supernatant liquid into a 150 ml beaker. Add 5 drops of Phenolphthalein Indicator solution and neutralize by the dropwise addition of approximately 0.5N Potassium Hydroxide. Add 3 ml of 50% Silver Nitrate and mix well.

5. Add 50 ml of cold (0-5°C) Reagent Alcohol saturated with Ag_2PO_3F.

6. Allow to stand in an ice bath for 20 minutes.

7. Filter through a single #41 filter paper or equivalent, transferring the bulk of the precipitate to the paper. Add 20 ml of cold (0-5°C) 80% reagent Alcohol saturated with Ag_2PO_3F to the beaker which contained the precipitate and dislodge any material clinging to the beaker with a rubber policeman. Transfer the precipitate to the filter paper. Repeat washing the beaker and precipitate with an additional 20 ml of cold 80% alcohol.

8. Add 0.5 $\pm$ 0.1 gram of Calcium Oxide to the sample in the paper. Transfer the filter paper containing the precipitate + CaO to a 30 ml Nickel dish using a rubber policeman and H_2O to aid in the transfer. Wash the beaker with about 10 ml of water adding the washings to the dish.

9. Mix well with a glass rod. Carefully wipe off the material clinging to the rod with a small piece of filter paper. Add the filter paper to the sample in the dish.

10. Place the dish on a steam bath first to dry, then under a heating lamp and evaporate to dryness and char. Finally ash in a muffle furnace maintained at 560-600°C for 1/2 hour. At no time should the sample be permitted to ignite and burn with a flame.

11. Cool to room temperature and transfer the main contents of the dish with about 30 ml of water to the fluoride distilling flask containing approx. 0.5 gm of glass wool.

12. Add about 10 ml of 70% Perchloric acid to the dish. Mix, and with the aid of water, transfer to the distilling flask. Add an additional 40 ml of the 70% Perchloric acid and 1 ml of Silver Perchlorate to the sample solution in the flask. Place a 500 ml volumetric flask under the distillate outlet.

13. Turn on the transformer connected to the heating mantle surrounding the steam generator and set at the highest dial reading (about 130). Leave the Hoffman clamp open until the water boils.

14. Turn on the transformer connected to the band heater and set the dial at 100.

15. When the temperature in the distilling flask is approximately 130°C and the water is boiling in the steam generator, steam is admitted to the distilling flask by closing the Hoffman clamp. The temperature in the distilling flask is maintained at 135° ± 2°C by temporarily turning off the transformer connected to the band heater if the temperature reaches 137°C and turning it on again if the temperature drops to 135°C. The installation of an automatic temperature control, known as the "Therm-O-Watch," to the band heater and thermometer of the distillation unit will allow the operator to perform other duties while the distillation is in progress. The "Therm-O-Watch" unit (Model S-6) is obtainable from Instruments for Research and Industry, 108 Franklin Avenue, Cheltenham, Pa.

16. Collect about 450 ml of distillate. Turn the unit off by opening the Hoffman clamp and shutting off both transformers.

17. Neutralize the distillate to litmus by the dropwise addition of 5% Sodium Hydroxide solution and dilute to volume with water at room temperature. Mix well.

18. Pipet a 10 ml aliquot of the neutralized distillate from step 17 into a 100 ml volumetric flask. Add 50 ml of water from a graduated cylinder. Using micro burets measure 5 ml of Eriochrome Cyanine R Solution and 5 ml of Zirconyl Chloride solution into the flask. Dilute the solution to volume with water and mix well.

19. Using 1 cm cells on the Beckman Spectrophotometer, measure the absorbance of the solution @ 527.5 mu versus the reference solution prepared as described below.

20. Refer to a standard curve prepared as described to obtain the milligrams of F equivalent. It is important that the reference solution be prepared in the same manner as in the preparation of the standard curve.

Calculations:

$$\frac{\text{(mg. F from curve) x 100}}{\text{(weight of sample in aliquot in grams x 1000) (step 18)}} = \text{\% F equivalent to } Na_2PO_3F$$

REFERENCE SOLUTION

Using the same buret described in the procedure, measure 3.00 ml of Eriochrome Cyanine R Solution into a 100 ml volumetric flask. Dilute to about 85 ml with water and add from a pipet 10 ml of 70/30 HCl solution. Dilute to volume with water and mix well. See Alternate Method for Total Fluoride, step 14.

PREPARATION OF STANDARD CURVE

1. Accurately weigh 0.8842 gms of NaF and transfer to a 1000 ml volumetric flask. Add 500 ml of distilled water and swirl to dissolve. Dilute to volume with water and mix well.

2. Pipet 10 ml of the solution from step 1 into a second 1000 ml volumetric flask. Dilute to volume with water and mix well. Each ml of this solution contains 0.0040 mg of F. Both solutions from 1 and 2 should be stored in polyethylene containers.

3. Using a semi-micro buret, deliver 5.00, 7.50, 10.00, 12.50 and 15.00 ml portions of the solution from step 2 into separate 100 ml volumetric flasks. Dilute to about 50 ml with water. Using the micro burets deliver 5 ml of Eriochrome Cyanine R and 5 ml of Zirconyl Chloride solutions into each flask. Dilute to volume with water and mix well.

4. Using 1 cm cells on the Beckman Spectrophotometer, measure the absorbance of each prepared solution at 527.5 millimicrons versus the reference solution as a blank.

5. Using K&E 359-14L graph paper, plot the observed absorbance as the ordinate and the milligrams F as the abscissa. Draw the best possible straight line through the points. Any point not falling on the line should be checked.

NOTE:

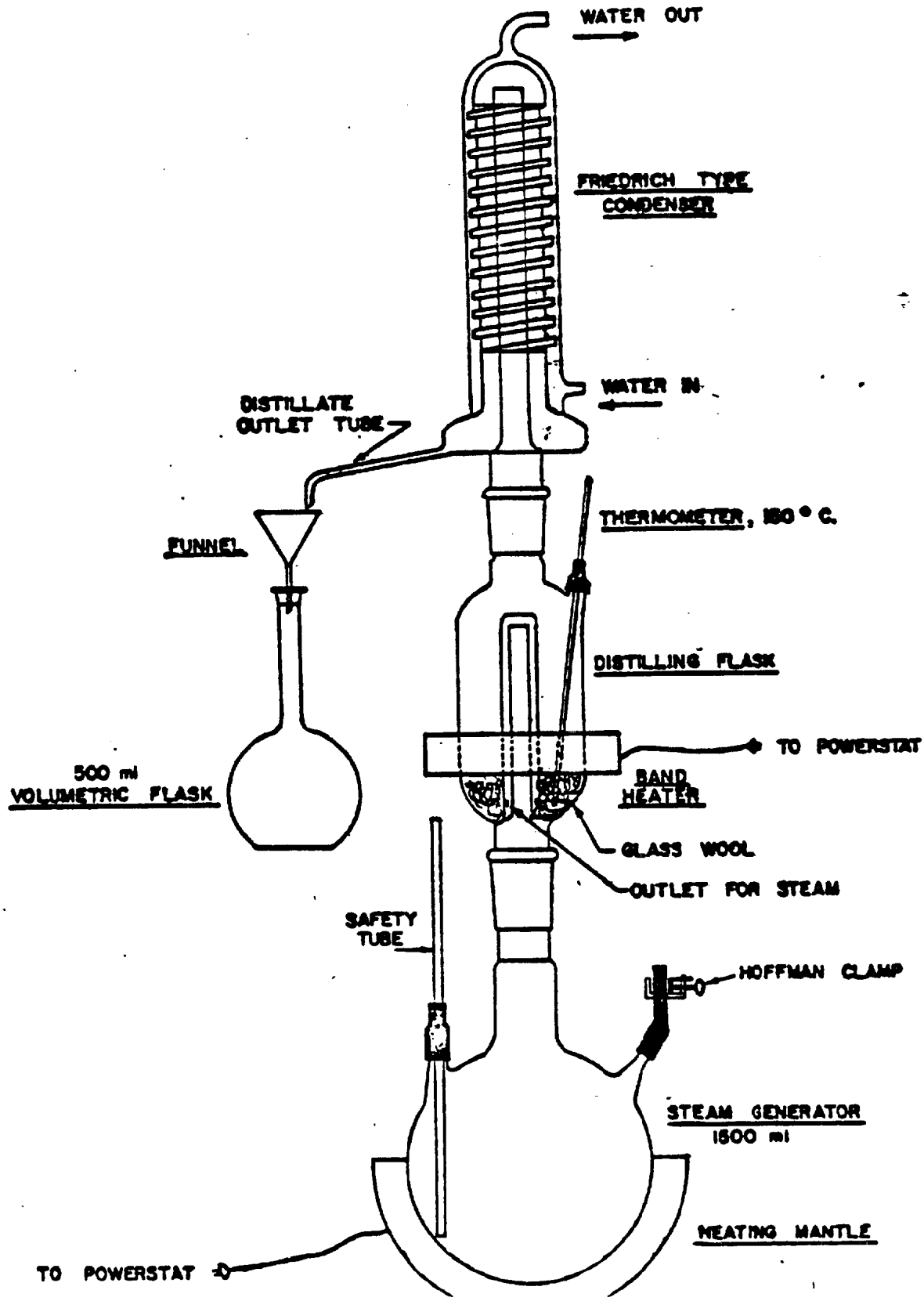
1. A new standard curve should be prepared each time new batches of dye or Zirconyl Chloride solutions are prepared.

2. To assure stability of reagents, at least two points on the standard curve should be checked frequently.

3. To remove possible contaminants, all glassware should be treated with hot 5% NaOH and washed thoroughly with distilled or deionized water.

FIGURE I.

FLUORIDE STEAM DISTILLATION APPARATUS



STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #22

TITLE: DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^{=}$ ION

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #22

DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^=$ ION

SCOPE

This procedure is applicable to the simultaneous determination of sodium monofluorophosphate and ionic fluoride in dental cream.

PRINCIPLE

The method is based on a modification of liquid chromatograph, combining ion exchange, eluent suppression and conductimetric detection.

APPARATUS REQUIRED

Dionex Analytical Ion Chromatograph
Analytical Column: 3 x 250 mm Chromex Anion Resin
Suppressor Column: 6 x 250 mm Chromax Anion Suppressor Resin
Eluent: 0.003 N Sodium Bicarbonate
0.002 N Sodium Carbonate
Flow: 230 ml/hr.

METHOD

A standard solution of MFP and sodium fluoride is used to calibrate the instrument. The sample is a 4% water soluble portion of a dental cream.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #23

TITLE: DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^=$ ION

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

DETERMINATION OF FLUORINE AS SOLUBLE $PO_3F^=$ ION

In addition to the previous methods, Fluorine as Soluble $PO_3F^=$ Ion can be calculated by subtracting the value obtained for Fluoride as Soluble F^- ion from the value obtained for Total Soluble Available Fluorine.

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #24

DETERMINATION OF SOLUBLE MONOFLUOROPHOSPHATE ION IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #24

DETERMINATION OF SOLUBLE MONOFLUOROPHOSPHATE ION IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Soluble monofluorophosphate ion is derived by subtracting soluble fluoride (ionic) from total soluble available fluoride.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #25

DETERMINATION OF FLUORIDE AS SOLUBLE F ION

Recommended for the following systems:

a. Sodium monofluorophosphate and silica abrasive.

TEST METHOD #25

DETERMINATION OF FLUORIDE AS SOLUBLE F ION

Principle

The toothpaste is extracted with water and the fluoride ion concentration of the extract is determined with a fluoride-specific ion electrode.

Apparatus

Corning Digital 112 Research pH/mV Meter.
Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.
Orion Single-Junction Reference Electrode 90-01.
Volumetric flasks, 25-ml, 50-ml, 1000-ml sizes.
Pipets, 1-ml, 2-ml, 3-ml, 5-ml, 8-ml and 25-ml sizes.
Polyethylene beakers, 100-ml.
Polyethylene bottles, 50-ml.
Semi-log graph paper, 1 cycle.
Centrifuge, Servall Type SP, Ivan Sorvall, Inc.
Centrifuge tubes, round bottom, polypropylene, 50-ml capacity.
Centrifuge tubes closure to fit 50-ml centrifuge tubes.
Buret, 50-ml.

Reagents

Sodium fluoride, ACS Reagent Grade. Prepare the solution shown below:
Weigh accurately 2.2105 grams of reagent grade sodium fluoride. Dissolve and dilute to 1 litre in water (1000 ug/ml F^- standard). Pipet 10 ml of 1000 ug/ml fluoride standard into a 1000-ml volumetric flask, dilute to volume with water and mix thoroughly. (10 ug/ml F^- standard). Transfer the solution to a polyethylene bottle for storage.
Sodium citrate dihydrate, Reagent Grade.
Sodium chloride, Reagent Grade.
Glacial acetic acid, Reagent Grade.
Buffer solution: Dissolve 147 grams sodium citrate dihydrate and 58 grams sodium chloride in 1000 ml water. Add 60 ml glacial acetic acid. Mix by stirring. Adjust with sodium hydroxide (approx. 10% solution) to pH 5.5. Cool the solution then make up to 2 liters with water, and mix well. Store in a polyethylene bottle.

Preparation of Standard Curve

Connect the lead of the fluoride electrode to the shielded input jack of the pH meter. Fill the reference electrode with the special filling solution supplied by Orion (No. 90-00-01). Do not use any other filling solution. Connect the lead of the reference lectrode to the reference input jack of the pH meter. Turn the meter onto STANDBY function and place the electrodes in distilled water for about 15 minutes to allow them to

equilibrate. Pipet 1 ml of 10 ug/ml fluoride standard and 25 ml buffer solution into a 50 ml volumetric flask, dilute to volume with water and mix well. Without using any additional water, transfer the contents of the flask into a clean 100 ml plastic beaker, draining the flask well. Carefully blot dry the electrodes with Kleenex tissues. Immerse the electrode tips in the buffered fluoride standard. Ensure that there are no air bubbles adhering to the electrode surface. Drop the stirring bar between them and stir at a moderate rate. Leave for 5-10 minutes until a constant potential is produced. Record the millivoltage. Remove the electrodes, rinse with distilled water and dry with Kleenex tissues. Determine the millivolt readings on the other standard with 2 ml, 3 ml, 5 ml and 8 ml of 10 ug/ml fluoride standard respectively. Plot the calibration graph on semi-log paper with the millivolt reading on the linear abscissa and concentration on the logarithmic ordinate.

Procedure

Weight about 2 grams of paste to an accuracy of ± 1 mg into a centrifuge tube. Add from the 50 ml buret small increments of distilled water, thoroughly mixing with a small glass rod after each addition until a smooth slurry is obtained. Continue the addition of water until the total volume added is 10 times the weight of the paste taken. Cap the tube and centrifuge at 4000-5000 rmp for 15 minutes. Immediately decant the supernatant liquid into a 50 ml polyethylene bottle. Pipet a 5 ml aliquot of the solution into a 50 ml volumetric flask. Add 25 ml of buffer solution, dilute to volume with water and mix well. Read the potential in millivolts, following the procedure used for the standard solutions.

Calculations

Determine the fluoride concentration in the sample solution by referring to the standard curve.

$$\%\ \text{Available Free Fluoride (1:10 dilution)} = \frac{I \times D \times 100}{W \times 5 \times 10^6}$$

$$= \frac{I \times D}{W \times 50000}$$

I = ug of fluoride read from the standard curve.

W = Weight of sample in grams.

D = ml of water used to prepare slurry.

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #26

DETERMINATION OF SOLUBLE (IONIC) FLUORIDE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #26

DETERMINATION OF SOLUBLE (IONIC) FLUORIDE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the soluble (ionic) fluoride in a sodium monofluorophosphate chalk dentifrice.

Reagents	Suggested Type or Source
Sodium Acetate	MCB Inc.
NaF	Baker Analytical Reagent
Polyethylene beakers	SGA
Orion Calomel Reference Electrode Solution	Orion Research Corp.

Apparatus	Suggested Type or Source
Specific Ion Meter Model 407-A	Orion Research Corp.

Procedure

Weigh accurately 10-15 gm $\pm$ 0.2 gm (w) of dentifrice into a 250 ml beaker and slurry with 20 ml of deionized water. Transfer quantitatively into a 100 ml volumetric flask and dilute to volume with deionized water. Mix, and pipette 10 ml of this solution into a 100 ml volumetric flask. Dilute to volume with sodium acetate solution (15% w/v) and mix thoroughly.

Standardize the specific ion meter by setting the center scale at 1 ppm fluorine and the upper end of the scale at 10 ppm fluorine.

Using the meter, determine the fluoride concentration in ppm (A) of the sample solution, immersing the specific electrode in about 40 ml of the solution contained in a polyethylene beaker.

Calculation

$$\text{Soluble (ionic) fluoride (ppm)} = \frac{A \times 1000}{W}$$

Where A = ppm fluorine from meter reading
W = weight of dentifrice in grams